HORTICULTURAL, MECHANICAL AND PHYSIOLOGICAL PROPERTIES OF ORANGES [*Citrus sinensis* (L.) Osb.] TREATED WITH GIBBERELLIC ACID

By

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Florida citrus growers and processors recently observed that gibberellic acid (GA_3) applied in the summer and fall sometimes increases orange juice yield by 2-10%. Such an increase could be of considerable economic benefit to growers whose fruits are valued according to soluble solids yield (% juice x juice Brix). Experiments were conducted to improve the efficacy of GA_3. Additionally, the hypotheses that GA_3 affects mechanical properties of peel or whole fruit such that juice extraction efficiency is increased, and that application reduces peel volume and thus increases juice yield by increasing the proportion of pulp in the fruit were tested.

Wash-off sprays within 1 to 2 h of GA_3 application reduced efficacy, suggesting that growers might consider re-application if rainfall occurs less than 2 h after treatment. Gibberellic acid applied at about color break increased juice yield of 'Hamlin' and 'Valencia' but not 'Pineapple' sweet oranges [*Citrus sinensis* (L.) Osb.]. Optimal juice yields were achieved when fruit were harvested about 2 months ('Hamlin') or 5 months

('Valencia') after GA_3 application. Juice Brix was sometimes reduced by GA_3 treatment, a finding that could be investigated further.

Gibberellic acid application before color break increased tensile strength, modulus of elasticity in tension, and shear strength of 'Valencia' orange peel by 10-20%, but had little effect on mechanical properties of the whole fruit. Moreover, there was no obvious relationship between mechanical properties of the peel or the whole fruit and juice yield. Thus, it is unlikely that GA_3 application increased juice yield by enhancing juice extraction efficiency. However, GA_3 treatment decreased peel volume and there was an inverse, linear relationship between peel volume and juice yield. Therefore, GA_3 probably increased juice yield by decreasing peel volume.

Gibberellic acid application slowed the loss of green peel color and reduced levels of soluble sugars in flavedo. In particular, fructose and glucose levels, were inversely related to green peel color. This suggests that one avenue of GA_3 action may have been to maintain green peel color by suppressing accumulation of hexose sugars.

CHAPTER 1
INTRODUCTION

More than 95% of the sweet orange [*Citrus sinensis* (L.) Osb.] crop in Florida is used for processing (Anonymous, 2001). The value of oranges for processing depends on solids yield (% juice x juice Brix), so practices that affect juice yield and Brix are important. Recent studies showed a positive relationship between applications of gibberellic acid (GA_3), a plant growth regulator, and juice yield (Davies et al., 1997; Davies et al., 2001) of sweet oranges but results were inconsistent and the basis for increased yield was unknown.

Inconsistent effects are a common disadvantage of plant growth regulators but management practices that improve efficacy can often be developed (Martin, 1983). Effects of GA_3 are dose-dependant (McDonald et al., 1987), so variables that affect uptake should be identified. Weather conditions should be considered when applying GA_3, because temperature and relative humidity affect drying of the delivery solution and uptake of GA_3 (El-Otmani et al., 2000; Greenberg and Goldschmidt, 1990). In Florida, rainfall might occur on a daily basis in late summer or fall, when GA_3 is often applied, but the effect of rainfall on GA_3 efficacy is unknown.

The efficacy of GA_3 with respect to peel senescence is related to the physiological age of the fruit and to the amount of time between application and harvest (Greenberg et al., 1992). Thus, application timing might also be important to optimize GA_3 effects on juice yield (Davies et al., 1999).

It is uncertain how GA_3 application increases juice yield. Most previous studies have found that GA_3 application has a marked effect on citrus peel quality and no effect on internal fruit quality (Coggins, 1969; Davies, 1986; El-Otmani et al., 2000). If GA_3 does not affect internal factors, then the observed increase in juice yields might relate to GA_3 effects on the peel. For example, some fruit processors believe that application of GA_3 improves fruit integrity such that juice extraction is more efficient and yield is thus increased (Albrigo and Carter, 1977; Davies et al., 1997). Alternately, GA_3 might reduce peel thickness and thus increase juice yield by increasing the proportion of pulp in the fruit. In order to achieve consistent increases in juice yield by GA_3 application, these hypotheses should be tested.

Most studies have found that application of GA_3 did not affect juice Brix (Coggins and Hield, 1968; Davies, 1986; El-Otmani et al., 2000) but others observed that application of GAs sometimes decreased juice Brix (Cary, 1975; Coggins and Hield, 1958; Monselise and Goren, 1965; Pozo et al., 2000). Because fruit for processing are valued according to juice yield and Brix, the potential effects of GA_3 on juice Brix should be tested.

Therefore, the objectives of this study were to test the following.

- Effect of simulated rainfall on GA_3 efficacy.
- Effect of GA_3 application and fruit harvest timing on juice yield and Brix.
- Effects of GA_3 on mechanical or physiological properties of the peel that might be related to juice yield.

CHAPTER 2
REVIEW OF THE LITERATURE

Gibberellic Acid is a Plant Growth Regulator

The striking effect of gibberellic acid (GA_3) on plant growth led to its discovery in 1926 (Kurosawa, 1926). *Gibberella fujikuroi* (Saw.) Wr. a pathogen of rice (*Oryza sativa* L.), produces large amounts of GA_3 as the end product of gibberellin (GA) metabolism (Sponsel, 1995). As the fungus colonizes rice, it secretes GA_3 that causes the stems to hyperelongate and the plant to lodge; a condition known as "foolish rice disease" (Phinney, 1983). Japanese scientists isolated GA_3 from the *Gibberella* fungus, and named the compound 'gibberellin' (Phinney, 1983). Then, it was shown that plants produce their own GA_3 and other GAs that function as hormones, stimulating many developmental processes including seed germination, stem elongation, vegetative bud break, and fruit set (Davies, 1995).

In plants, gibberellins are synthesized in the plastid (Heldt, 1997) and originate from mevalonic acid that forms isopentenylpyrophosphate (IPP), the C_5 subunits of terpenes including GAs (El-Otmani et al., 1995). Prenyl transferases catalyze four successive head-to-tail condensations of IPP that produce geranylgeranyl pyrophosphate, a C_{20} intermediate that undergoes cyclization reactions to yield GA_{12}-aldehyde, the first-formed GA (Sponsel, 1995). The *ent*-gibberellane ring system is amenable to structural modification, and more than 100 different GAs have been identified in plants and certain fungi (Sponsel, 1995). Of the numerous identified GAs, only a few are known to be

biologically active; the others are probably intermediates or conjugates of active forms (Kende and Zeevart, 1997).

In citrus, more than 20 different GAs have been identified (El-Otmani et al., 1995). The most abundant GA in citrus is GA_{29}, which occured in all tissues tested, as did GA_8 and GA_{20} (El-Otmani et al., 1995). Gibberellin $_{29}$ and GA_8 supposedly lack biological activity (Reeve and Crozier, 1975; Smith, 1992), but El-Otmani et al. (1995) speculated that the abundance of GA_{29} in citrus may indicate that it can influence the levels of other GAs. Alternately, GA_{29} might be the most common intermediate or conjugate of biologically active GAs in citrus. Gibberellic acid occurs naturally in citrus, but not as frequently as other bioactive GAs such as GA_1 (El-Otmani et al., 1995). The presence of certain GAs is developmentally dependant, but the physiological significance of this fact is not known (El-Otmani et al., 1995). Citrus organs that are particularly rich in GAs include vegetative shoots, leaves and developing fruit (El-Otmani et al., 1995), as observed in other plants (Hedden and Kamiya, 1997; Sponsel, 1995).

Gibberellic Acid Application in Citriculture

Soon after the discovery of GA_3, an efficient fermentation process was developed that used *Gibberella fujikuroi* to produce commercial quantities of relatively inexpensive GA_3 (Martin, 1983). The new availability of GA_3 coincided with an increased interest in agricultural chemicals in general and plant growth regulators in particular (Martin, 1983). Therefore, many scientists applied GA_3 to horticultural crops, including citrus, to determine if it could improve quality or yield.

Citrus fruit are a unique type of berry called a hesperidium (Schneider, 1969). Their leathery peel is composed of two anatomically distinct layers known as the flavedo and albedo. The flavedo, the outer, colored-portion of the peel, consists of a cuticle-

covered epidermis and a few layers of tabular, thick-walled cells interspersed with oil glands (Albrigo and Carter, 1977). The albedo, the white, inner portion of the peel, consists of several layers of branched, thin-walled cells, numerous intercellular air spaces, and a few oil glands. Albedo thickness may vary widely among citrus species and cultivars but, except for certain mandarins, the albedo typically accounts for most of the peel thickness (Spiegel-Roy and Goldschmidt, 1996). The peel surrounds 10-14 ovarian locules ('segments') that are arranged around an axis of spongy white tissue that is anatomically similar to the albedo (Albrigo and Carter, 1977).

The application of GA_3 to citrus has many interesting effects on reproductive physiology that are of commercial value. Application of GA_3 in the spring can improve fruit set, especially of parthenocarpic mandarins (*Citrus reticulata* Blanco) (Coggins, 1981), but the most widespread use of GA_3 in citrus is to delay peel maturation and thus reduce the incidence and severity of senescence related peel disorders (Coggins and Hield, 1968; Davies, 1986). When GA_3 is applied in the fall to delay peel maturation, it might also reduce or delay flowering; an effect that can be useful or undesirable, depending on the circumstances.

Gibberellic Acid and Peel Maturation

Citrus fruit are nonclimacteric. Because of their slow maturation, both fresh and processing fruit may be "stored" on the tree for several months after fruit has reached legal maturity until it is financially desirable to harvest (Coggins, 1969; Davies and Albrigo, 1994). Generally, such storage does not decrease internal quality; however, the peel of non-GA_3 treated fruit may deteriorate because peel senescence sometimes begins before fruit has attained internal maturity (Coggins, 1968). In the fall, cooler temperatures induce peel color break, a green to yellow color change of the flavedo (the

epicarp, or outer layer of the peel) as chloroplasts are converted to chromoplasts (Coggins and Jones, 1977; Erickson, 1968). During this time, peel puncture resistance (PPR) gradually declines (Coggins and Lewis, 1965). The commencement of color break and the decline of PPR characterize citrus peel maturation.

The length of time that fruit may be stored on the tree depends, in part, on the rate of peel degradation (Coggins, 1968; Davies, 1986). As the peels senesce, they lose their integrity, and are more susceptible to mechanical damage (Coggins, 1981; Ritenour and Stover, 1999). Senescent peels also develop physiological disorders, such as creasing (Monselise et al., 1976), puffing (Garcia-Luis et al., 1985), stickiness, and rind staining, which reduce marketability of fresh fruit (Coggins and Hield, 1968; Davies, 1986). Senescent peels are typically thought of as a fresh fruit problem (Davies, 1986), but fruit that are mechanically damaged during harvest or those with overly soft or deformed rinds are also culled before processing (Braddock, 1999; Davies et al., 1997). Therefore, it is desirable to delay peel maturation to extend the harvest season (Dinar et al., 1976).

In the 1950s it was discovered that GAs had the potential to delay citrus peel maturation. Coggins and Hield (1958) found that dipping green fruit of 'Thompson Improved' navel orange [*Citrus sinensis* (L.) Osb.] into a solution of potassium gibberellate (KGA) delayed color break. Later it was shown that solutions of KGA applied to trees with a sprayer also delayed color break of 'Washington' navel (Coggins and Lewis, 1965) and 'Valencia' oranges (Coggins et al., 1960b), grapefruit (*Citrus paradisi* Macf.) (Coggins et al., 1962), and lemons [*Citrus limon* (L.) Burm. f.] (Coggins et al., 1960a). It is widely accepted that GA_3 delays (Davies, 1986; El-Otmani et al., 2000; Wilson, 1983), and may even partially reverse citrus peel senescence (Monselise,

1977). In fact, citrus peel cells are so sensitive to GA_3 application, that they may be used as a bioassay for gibberellins (Eilati et al., 1969; Henning and Coggins, 1988). Gibberellins have long-lasting effects on peel color (up to 8 months) and green fruit are undesirable at harvest, so the likely harvest date must be considered before application (Coggins, 1981; Coggins et al., 1965)

Treatment with GA_3, as observed for green peel color, slows the decrease of peel puncture resistance (Coggins, 1968; Davies, 1986; Wilson, 1983). Peel puncture resistance (PPR) is an indicator of the physical integrity of citrus peels (Coggins and Lewis, 1965). The physiological basis of GA_3 effects on peel integrity is poorly understood. Coggins and Lewis (1965) used probes of different diameter to puncture citrus peel and determined that cutting forces were needed to overcome PPR. They also suggested that the structure of the cuticle, epidermis, and the upper flavedo cells probably contributed to puncture resistance. A histological study of the peel of fruit treated with GA_3 showed that cells in peels treated with GA_3 are more numerous and are packed closer together, with less cell wall degradation compared with peel cells of untreated fruit (Coggins and Hield, 1968). Thus, the peel of GA_3-treated fruit can be described as being more "compact" compared with untreated peel.

Muramatsu et al. (1999) speculated that peel firmness was related to the concentration of cell wall polysaccharides. Nagar (1994) proposed that high levels of GA_3 might suppress expression of genes related to cell wall degradation. Moreover, silver thiosulphate, an inhibitor of ethylene action, retarded citrus peel softening. This suggests that GA_3 might inhibit softening by interfering with ethylene action (Nagar, 1994). Iglesias et al. (2001) proposed a similar hypothesis to explain GA_3 effects on peel

color, but it should be remembered that citrus fruit are non-climacteric and thus produce very low levels of ethylene (Eaks, 1970).

Gibberellic Acid and Flowering

Gibberellins are the only plant growth regulators that have a consistent, reproducible effect on citrus flowering (Davies and Albrigo, 1994; Spiegel-Roy and Goldschmidt, 1996). Late fall application of GA_3 may inhibit flower induction of citrus and thus reduce or delay subsequent flowering (Davenport, 1990; Monselise and Halevy, 1964). Application of GA_3 may also have an indirect effect on flowering because the presence of fruit stored on the tree, especially fruit with green colored peels, may inhibit flowering (Garcia-Luis et al., 1986). In addition to delayed or reduced flowering, GA_3 application favors production of leafy vs. leafless inflorescences (Goldschmidt and Monselise, 1972; Guardiola et al., 1977). Therefore, potential effects of GA_3 on flowering must be considered when applying GA_3 in the fall and winter (El-Otmani et al., 2000).

Reduced flowering caused by GA_3 application can be desirable or undesirable. Certain citrus cultivars have an alternate bearing problem. In "on" years, these trees produce a large crop of small fruit, but in "off" years they produce small crops or no crop at all (Lewis et al., 1964). Application of GA_3 in the fall preceding an "on" year may reduce flowering and disrupt the alternate bearing cycle (Agusti et al., 1981; Moss and Bellamy, 1972). However, application of even relatively small amounts of GAs may drastically reduce flowering (Spiegel-Roy and Goldschmidt, 1996), so fall or winter GA_3 application might reduce yield the next year.

Variables that Influence Efficacy of GA_3

Uptake of Gibberellic Acid

The efficacy of GA_3 on citrus peel is dose dependent (McDonald et al., 1987). Therefore, the physiology of GA_3 uptake by citrus fruit is of considerable interest, but some factors that affect uptake remain unclear (Coggins et al., 1992). The concentration of GA applied, atmospheric humidity and temperature, pH of the delivery solution and the use of surfactants can all affect GA uptake.

Citrus peel absorbed GA_3 at a faster rate when the delivery solution was allowed to dry out than when it was continuously wet, probably because drying concentrated the solution, whereas the continuously wet treatment became less concentrated as GA_3 was absorbed by the fruit (Greenberg and Goldschmidt, 1990). However, the GA_3 uptake rate was greater at high humidity than low humidity when the solution was allowed to dry (Greenberg and Goldschmidt, 1990). Apparently, the "dry" GA_3 residue acts as a concentrated solution at high humidity. Although GA_3 is lipophillic, enhanced uptake at high humidity suggests that hydrophilic processes are also involved in uptake of GA_3.

Acidification of the delivery solution has an inconsistent effect on GA_3 uptake. In Israel, acidification increased GA_3 efficacy (Greenberg et al., 1984; Greenberg and Goldschmidt, 1988), regardless of the acidifying agent used (Greenberg and Goldschmidt, 1988). An acidic delivery solution should make GA_3 more effective by favoring the anionic form (Greenberg and Goldschmidt, 1989). In contrast, testing in California and Florida showed that delivery solutions of low pH did not improve GA_3 efficacy (Coggins et al., 1992; Davies et al., 2001), though efficacy was reduced by an alkaline solution (Coggins et al., 1974). Petracek (pers. com.) observed that some

adjuvants degrade at low pH and thus might become ineffective. This could partly explain why acidification did not improve efficacy in the Florida study .

Surfactants or adjuvants improved efficacy of GA_3 in several studies (Coggins et al., 1992; Greenberg et al., 1987; Greenberg and Goldschmidt, 1990; Henning and Coggins, 1988). The surfactant L-77 (Silwet®) was so effective at enhancing GA_3 uptake that 10 times the concentration of GA_3 had to be used in the absence of L-77 to achieve a comparable effect (Greenberg, 1987). However, Davies et al. (2001) found that L-77 did not improve GA_3 efficacy. The efficacy of various adjuvants was not necessarily related to their ability to decrease surface tension (Henning and Coggins, 1988). Instead, some adjuvants might increase uptake of GA_3 by selectively dissolving certain cuticular waxes (Greenberg and Goldschmidt, 1988). Perhaps adjuvant efficacy is related to differences in the cuticle chemistry of fruits grown in different regions. Because the efficacy of surfactants varies widely, local performance tests should be conducted (El-Otmani et al., 2000).

Laboratory research indicates that peak uptake of ^{14}C-GA_3 is within 1 h of application, but may continue for hours or days (Ferguson et al., 1986). It is unknown whether the persistent effects of GA_3 on citrus fruit might be attributed to slow metabolism, prolonged uptake, or both. At least 20% of applied ^{14}C-GA_3 remained in the peel of 'Shamouti' orange 5 d after treatment (DAT; Goldschmidt and Galili, 1974), and 1% remained 100 DAT (Goldschmidt and Galili, 1981), suggesting that GA_3 was metabolized slowly. However, nearly 70% of the applied compound remained on the surface of the fruit 1 DAT, while only 2% had penetrated the flavedo by this time (Goldschmidt and Galili, 1981).

Residual GA_3 on the fruit surface might continue to penetrate the peel and thus enhance efficacy, but there are no data to confirm this. In Florida, GA_3 is often applied in the late summer or fall, when rainfall may occur on a daily basis. Rainfall might remove or dilute applied GA_3, thus reducing efficacy, but field data are lacking.

Timing of Gibberellic Acid Application and Fruit Harvest

The degree to which GA_3 retards peel senescence is related to the physiological age of the fruit and the amount of time between application and harvest, so the timing of GA_3 application might be critical for optimal efficacy (Greenberg et al., 1992). Color break is often used as a reference to recommend timing of GA_3 sprays because it approximately corresponds with the onset of peel degradation that is slowed, but not reversed by GA_3 application (Coggins and Hield, 1968). For example, when GA_3 was applied before color break, peels retained greater integrity than when GA_3 was applied after color break (Coggins and Lewis, 1965). Similarly, when GA_3 was applied to navel oranges after color break, PPR was greater than non-sprayed fruit at harvest, but less than that of fruit sprayed before color break (Coggins, 1969). Similarly, Gregoriou et al. (1996) found that GA_3 application time affected water spot incidence on mandarins, a peel disorder which is also related to peel integrity. Therefore, the effect of GA_3 applied before, during, and after color break should be tested when evaluating effects of GA_3 application on other variables.

Potential for Gibberellic Acid Application to Increase Juice Yield

Since the earliest testing of GA_3 on citrus, there have been occasional reports that GA_3 application increased juice yield of oranges by 3-5% (Coggins and Hield, 1958; Coggins, 1969; Coggins, 1981). However, this effect was uncommon and inconsistent, and the juice yield of fresh fruit is of limited commercial significance, so effects on juice

content did not receive much attention (Coggins and Henning, 1988). Eventually, after most studies reported no effects of GA_3 application on internal quality, it was generally accepted that GA_3 application only affected the peel and most recent studies have not considered GA_3 effects on internal quality (Coggins and Hield, 1968; Coggins and Henning, 1988; Davies, 1986; El-Otmani et al., 1995; El-Otmani et al., 2000; Spiegel-Roy and Goldschmidt, 1996).

Nevertheless, the successful use of GA_3 to extend the harvest season of fresh citrus fruit recently prompted Florida citrus growers and processors to test the effects of GA_3 on processing fruit (Davies et al., 1997). Initially, the goal was to use GA_3 to improve the integrity of fruit stored on the tree to extend the harvest season, but GA_3-treated fruit sometimes yielded up to 10% more juice than non-treated fruit, even when harvested early (Davies et al., 1997; Davies et al., 1999; Davies et al., 2001). Such an increase would be of considerable economic benefit to Florida citrus growers because processed fruit value increases with juice yield and Brix (Braddock, 1999). Therefore it is desirable to determine why GA_3 application increased juice yields and to develop management practices for optimization of juice yield by GA_3.

Application of GA_3 has a variety of affects on tree and fruit physiology that could improve juice yield. For example, supplemental GA_3 increased xylogenesis in fruitlets (Guardiola et al., 1993) and increased fruit stem diameter (Krezdorn and Cohen, 1962). These similar responses could improve water transfer from tree to fruit and thus increase juice yield. However, relatively high levels of GA_3 ($500\ mg \bullet L^{-1}$) were used when fruit stem diameter was increased (Krezdorn and Cohen, 1962). Today, GA_3 is applied at less

than half that concentration and there are no contemporary data to show that smaller amounts of GA_3 affect fruit stem diameter.

Coggins (1981) reported that application of GA_3 appeared to stimulate wax deposition by peel epidermal cells, and Fucik (1981) found that GA_3 treatment reduced weight loss of grapefruit in storage. However, a later study found that GA_3 application slowed deposition of epicuticular wax (El-Otmani and Coggins, 1985), and others found that GA_3 did not affect fruit weight loss in storage (El-Otmani and Coggins, 1985; El-Otmani et al., 1986). Therefore it seems unlikely that GA_3 treatment increased juice yield by decreasing water loss.

Recently it was suggested that improved juice yield of GA_3-treated oranges might be related to treatment effects on mechanical properties of the peel or fruit (Davies et al., 1997; Fidelibus et al., 2002b; J. Keithly, pers. com.). Most juice processors in Florida use extraction machines that squeeze fruit between two inter-digitating cups (Braddock, 1999). Cutters in each cup punch holes in the fruit that allow the peel to be extruded through the top cup while the pulp enters the juice stream through the bottom cup (Berry and Veldhuis, 1977). If fruit are excessively soft or misshaped the top cup might impact the fruit at an oblique angle and thereby reduce extraction efficiency (Albrigo and Carter, 1977). Thus, the extraction subjects the peel or fruit or both to compression, shear and tensile forces. It was postulated that GA_3-treatment effects on these mechanical properties might enhance juice extraction efficiency. In support of this hypothesis, Davies et al. (2001) reported a significant positive correlation between PPR and juice yield ($r^2 = 0.58$) but the effect of GA_3 treatment on basic mechanical properties of citrus peel and fruit is unknown.

Mechanical Properties of Citrus Peel and Fruit

Basic mechanical property data of citrus peel and fruit are limited, despite the fruit's economic importance. The complex structure of citrus fruit might partly explain why mechanical property data are limited and why most mechanical property data are of pieces of citrus peel or flesh, instead of whole fruit. Ahmed et al. (1973) reported shear stress data of peel pieces collected on one harvest date. Kaufmann (1970) reported that the citrus peel became more extensible when water potential or temperature of the peel decreased. Gyasi et al. (1981) determined Poisson's ratio for citrus fruit peel and pulp but did not publish the moduli used in calculations. Churchill et al. (1980) conducted rupture and tensile tests on peel pieces and whole fruit, but reported data as maximum force and deformation instead of stress and strain. Miller (1986) determined stress index, modulus of elasticity and rupture force of freeze-damaged and non-damaged mandarins, oranges and grapefruit. Sarig and Nahir (1973) reported the initial and permanent deformations of a creep test of citrus fruit to indicate firmness.

There are many reports that GA_3-treatment improves 'firmness' of citrus fruits (Coggins, 1965; Davies, 1986), but horticulturists generally consider fruit puncture resistance and firmness as synonyms (Harker et al., 1997). Because the peel of fruit treated with GA_3 is more resistant to puncture than non-treated fruit, this treatment might make fruit less susceptible to mechanical damage (Davies et al., 1997; Ritenour and Stover, 1999), but mechanical properties data of fruit treated with GA_3 are lacking.

Greenberg et al. (1992) attempted to measure the effect of GA_3 treatment on fruit 'softness' by placing a weight on 'Minneola' tangelo fruits treated with GA_3 and non-treated and measuring the deformation of fruit diameter 30 s after the weight was applied and again 30 s after the weight was removed. Deformation was less for fruit treated with

GA_3 than for non-treated fruit, and GA_3 treated fruit recovered more deformation than the non-treated fruit. From these data, the authors suggested that fruit treated with GA_3 were less soft than non-treated fruit. However, their improper methodology limits the usefulness of the data. Because they subjected the fruit to a static weight over time, the test was more a measure of creep than softness. In addition, the authors did not measure or estimate contact area between the weight and the fruit, and deformation was not expressed as a percent of fruit diameter. In fact, they did not measure fruit diameter. No other attempts to measure effects of GA_3-treatment on basic mechanical properties of citrus peel or fruit have been reported.

Gibberellins and Peel Growth

If application of GA_3 does not affect juice extraction efficiency, then it must increase the amount of juice in the fruit or the pulp:peel ratio. Most studies have found that internal fruit quality, including juice yield and Brix, was not affected by GA_3 application (Coggins, 1968; Davies, 1986). Because GAs stimulate growth of juice vesicles in vitro (Altman et al., 1982; Harada et al., 2001), but exogenously applied GA_3 rarely affects internal quality, it seems likely that applied GA_3 must not diffuse across the peel in physiologically active levels (Goldschmidt, 1983). This hypothesis is supported by observations that ^{14}C-GA_3 applied to the peel was recovered from the albedo and flavedo tissue, but not from the juice or seeds (Ferguson et al., 1986; Greenberg et al., 1987). However, the effect of GA_3 treatment on peel growth is unclear. Given the substantial effects of GA_3 on peel physiology, it seems that GA_3 might increase juice yield indirectly by controlling peel growth or the capacity for phloem transport into fruit and unloading in the inner peel.

Reports of GA_3 effects on peel growth are variable and appear to depend on the amount and duration of exposure to GAs. For example, 'Shamouti' oranges in Israel sometimes have very thick, coarse peel tissue caused by excessive cell proliferation, and this condition is associated with abnormally high levels of endogenous gibberellins (Erner et al., 1976; Monselise, 1977). Likewise, Goldschmidt (1983) applied gibberellin A_{4+7} in lanolin paste to grapefruit and 'Shamouti' orange peel and observed that cells in the vicinity of the paste proliferated to form a thick, coarse peel. In fact, the excessive peel growth physically constrained growth of sub-adjacent juice vesicles. Coggins et al. (1960b) found that increasing the concentration of KGA applied to 'Valencia' oranges increased peel thickness and decreased juice yield. In contrast, Ferguson (1984) found that GA_3 applied to grapefruit increased juice content.

A single application of GA_3 in the fall or winter sometimes results in fruit with a thinner peel than non-treated fruit (Coggins and Hield, 1958; Coggins and Hield, 1968; Pozo et al., 2001; Garcia-Luis et al., 1985). Gibberellic acid is especially effective at constraining peel growth of mandarins that, unlike oranges, normally develop a puffy peel as they senesce (Pozo et al., 2001; Garcia-Luis et al., 1985). Whether gibberellin maintains existing peel cells, or causes peel cell proliferation, the result is more viable cell walls in peel tissue (Coggins and Hield, 1968). In any case, peel thickness is not necessarily correlated with juice yield (Fidelibus et al., 2002a).

Application of GA_3 might alter water exchange between the fruit and tree, as proposed by Garcia-Luis et al. (1985), or reduce evaporation from the fruit surface (Fucik, 1981). If either hypothesis is correct, then the peel of GA_3-treated fruit might be expected to have a higher water content than the peel of non-treated fruit because water is

primarily drawn from the peel rather than the pulp (Rokach, 1953). The water status of the peel might be inferred by the gap between the cut edges of the peel that results when fruit are sliced mid way through the equator (Kaufmann, 1970).

Physiology of Gibberellic Acid and Peel Color

In the fall or winter, the colored portion of the peel (flavedo) of sweet orange fruit changes color from green to yellow. This process, known as color break, involves conversion of chloroplasts to chromoplasts and is stimulated by low temperatures (Erickson, 1968).

The physiology of color break is not fully understood but it appears to be modulated by peel sugars (Goldschmidt and Koch, 1996). For example, low temperature (5° C) stimulated invertase activity and increased levels of reducing sugars in grapefruit flavedo (Purvis and Rice, 1983). Increased levels of soluble sugars also corresponded with color break of 'Valencia' orange peel (Huff, 1984). Moreover, peel pieces in culture degreened when sucrose levels of the media were increased and regreened when sucrose was not provided (Huff, 1984). Likewise, regreening of 'Valencia' orange occurs in the spring (Thompson et al., 1967) and is preceded by a decrease in peel soluble sugars (Huff, 1984). Recently, it was demonstrated that peel sugar levels could be increased and color break advanced by injection of sucrose into phloem subtending the fruit (Iglesias et al., 2001). All of these observations are consistent with the theory that high levels of soluble sugars down-regulate genes encoding photosynthetic products (Koch, 1996).

Endogenous hormones and exogenous applications of a similar plant growth regulator are not necessarily functionally equivalent (Davies, 1995). However, there is evidence that endogenous gibberellins and exogenous applications of GA_3 have the same

effect on peel color. Eliati et al. (1969) noted that gibberellins were associated with the chloroplast and thus might be related to chloroplast integrity. This is true, but it is now known that terpenes, including GAs are produced in the plastid (Heldt, 1997) so the association between GAs and the chloroplast might not imply a protective role. The observations that endogenous GAs decreased as green peel color decreased (Monselise, 1977), and increased prior to regreening of 'Valencia' oranges in the spring (Rasmussen, 1973) do suggest that endogenous GAs help maintain chloroplasts.

The mechanism by which GA_3 delays color break is unknown, but it seems likely that it might affect soluble sugar levels of the peel or the ability of peel tissues to sense sugars. There are precedents for growth regulators to have both effects. For example, GA_3 applied to *Fuchsia hybrida* suppressed flowering and reduced apex sucrose levels that stimulate flowering (King and Ben-tal, 2001). Likewise, auxin inhibited sucrose modulation of sugar sensitive genes of soybean (DeWald et al., 1994). In a sucrose feeding study, Iglesias et al. (2001) found that GA_3 treatment did not affect peel color in the absence of an elicitor such as sucrose. The authors suggested that GA_3 treatment might act by repressing an ethylene response that is stimulated by high sucrose levels. However, GA_3 treatment might also have affected flavedo sucrose levels, or levels of other carbohydrates such as glucose or fructose might have been altered by sucrose feeding.

Recently, it was shown that GA_3 application timing affected peel color and juice Brix of sweet oranges such that the application times that best maintained green peel color also caused a reduction in juice Brix (Fidelibus et al., 2002b). It is unknown how GA_3 treatment could affect juice Brix, but as in the case of peel color, sugar levels might

be involved. In grapefruit, sugars followed an ascending gradient from peel to pulp until about color break and a descending gradient thereafter (Koch and Avigne, 1990). Therefore, high sugar levels in the peel might aid sugar accumulation in the pulp. If GA_3 treatment reduced levels of peel sugars, then peel color and juice Brix could both be affected. The potential relationship between GA_3 effects on peel sugar levels and juice Brix could be further investigated.

In summary, the application of GA_3 has the potential to increase juice yield of oranges. However, research is needed to improve efficacy and to understand the mechanism by which GA_3 application increases juice yield. Moreover, the possibility that GA_3 application might produce undesirable results, such as reduced flowering or lower juice Brix, should also be investigated.

CHAPTER 3
GIBBERELLIC ACID WASH-OFF STUDIES ON 'HAMLIN' ORANGE

Introduction

Growers apply gibberellic acid (GA_3) to citrus in the summer or fall to delay peel maturation and thus reduce the prevalence and/or severity of senescence-related peel disorders of fresh fruit (Davies, 1986) or to increase juice yield of processing oranges (Davies et al., 1997; Davies et al., 2001). Efficacy of GA_3 is positively correlated with the amount absorbed (Henning and Coggins, 1988). The physiology of GA_3 uptake is still largely unknown, but efficacy may be enhanced by surfactants, acidic delivery solutions, and high relative humidity (Coggins et al., 1992; Gilfillan, 1986; Greenberg and Goldschmidt, 1990).

Peak uptake of ^{14}C-GA_3 by the peel is within 1 h of application but uptake may continue for hours or days (Ferguson et al., 1986). It is unknown whether the persistent effects of GA_3 on citrus fruit can be partly attributed to prolonged uptake. Because GA_3 is often applied in the late summer or fall, when rainfall may occur on a daily basis in Florida, possible effects of post-application rainfall on efficacy should be examined. Therefore, research was conducted to determine whether there was a relationship between GA_3 efficacy (in terms of effects on peel hue angle and peel puncture resistance) and time between GA_3 application and wash-off.

Materials and Methods

Fourteen or 15-year-old 'Hamlin' orange [*Citrus sinensis* (L.) Osb.] trees on sour orange (*Citrus aurantium* L.) rootstock located in a research grove on the University of Florida, Gainesville campus were used in this study. On 22 Oct. 1998 and 7 Oct. 1999, 10 L of a GA_3 (ProGibb®, Valent BioSciences; 45 g ai/acre) and Silwet® (0.05% v/v) solution was applied to 15 (1998) or 18 (1999) trees. The GA_3 was applied between 0700 and 0900 HR with a backpack sprayer (Solo Inc., Newport News, VA). Each hour, for the next 4 (1998) or 5 (1999) h, three different GA_3-treated trees were sprayed with 20 L of water to simulate rainfall (ca. 0.005 m of H_20 applied to area under canopy). Three additional trees did not receive a GA_3 treatment (wash-off at time 0) and three trees were sprayed with GA_3 but not with water. Air temperature in the tree canopy was measured with a HoboPro data logger (Onset Computer Corp., Bourne, Mass.).

Ten fruit were randomly harvested around the perimeter of each tree (between 1 and 2 m height), on 24 Nov. 1998 and 8 Jan. 1999 and 29 Nov. and 21 Dec. 1999. Peel color (hue angle) was measured with a Minolta chroma-meter (Minolta Inc., Ramsey, NJ). Peel puncture resistance (PPR) was measured with an Accuforce force gauge (Ametek, Largo, FL). Color data were not collected on 24 Nov. 1998 due to a malfunction of the chroma-meter. Average hue angle and PPR for each fruit were determined from three measurements per fruit (equidistant around the fruit equator). Data reported for each tree are the average hue angles and PPRs of the 10 fruit sample. Data were subjected to regression analyses to determine the relationship between peel variables and time until wash-off.

Results and Discussion

Quadratic or cubic models fit certain data fairly well with respect to r^2 values (models not shown), but those models have limited practical use in this study because they lack a biological explanation and are not supported by laboratory findings. Instead, a linear plateau model was fitted to each data set (Figs. 1-1 to 1-4). This model assumes a linear relationship between the dependant and independent variables until a join-point after which the relationship between the variables plateaus. In this study, the join-point represents a time after application where subsequent rainfall might have little effect on GA_3 efficacy. The initial slope and the join-point of peel hue angle and PPR data were similar for harvests of the same year (according to students' t test; $P \leq 0.05$), so data from one harvest per year are displayed (Figs. 1-1 to 1-4).

In 1998-99, the wash-off treatments generally had little affect on peel hue angle or PPR (Fig. 1-1). A plateau model fit the 8 Jan. 1999 peel hue data well ($r^2 = 0.75$), however, the steep slope and low join-point (0.59 h) are not in agreement with most of the other data. Peel puncture resistance data of 8 Jan. 1999 suggests a less steep slope and a later join-point (1.15 h) than peel hue data; however, the PPR data do not fit a linear plateau model as well as the peel hue data ($r^2 = 0.34$; Fig. 1-2).

Peel hue and PPR suggest that in 1998 most GA_3 uptake occurred within 1 h of application, or that wash-off treatments were ineffective at removing or diluting GA_3. Laboratory research also showed that uptake of $^{14}C\text{-}GA_3$ is most rapid in the first hour following application (Ferguson et al., 1986), and Gilfillan and Cutting (1992) suggested that GA_3 efficacy was reduced by run-off following application. Moreover, trees that

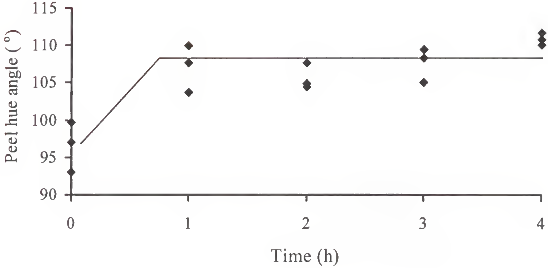

Fig. 1-1. Relationship between peel color (hue angle) of 'Hamlin' oranges and time between application of GA_3 and wash-off spray, 8 Jan. 1999. Fruit were treated on 22 Oct. 1998. Each point represents the mean of a 10-fruit sample selected from a single tree. Model y = 96.87 + 19.07x, join-point = 0.59, r^2=0.75.

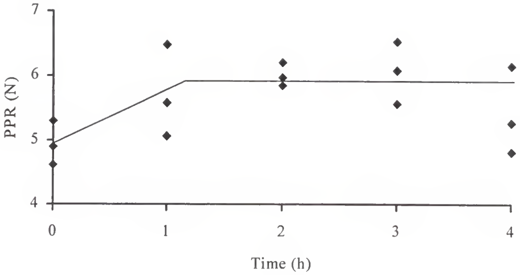

Fig. 1-2. Relationship between peel puncture resistance (PPR) of 'Hamlin' oranges and time between application of GA_3 and wash-off spray, 8 Jan. 1999. Fruit were treated on 22 Oct. 1998. Each point represents the mean of a 10-fruit sample selected from a single tree. Model $y = 4.94 + 0.77x$, join-point = 1.56, $r^2 = 0.34$.

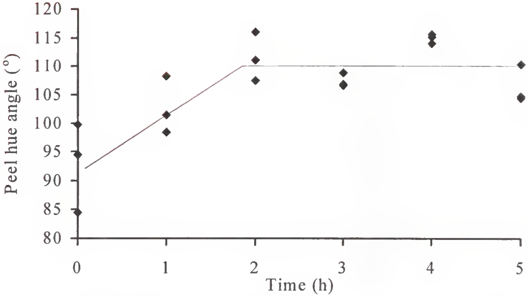

Fig. 1-3. Relationship between peel color (hue angle) of 'Hamlin' oranges and time between application of GA_3 and wash-off spray, 29 Nov. 1999. Fruit were treated on 7 Oct. 1999. Each point represents the mean of a 10-fruit sample selected from a single tree. Model $y = 92.87 + 9.8x$, join-point = 1.78, $r^2 = 0.68$.

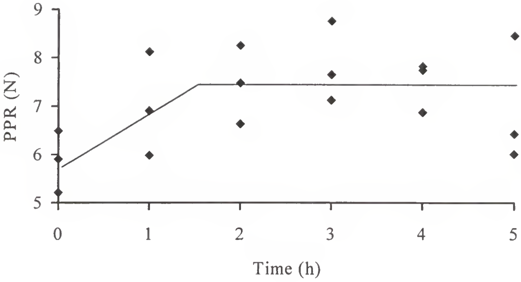

Fig. 1-4. Relationship between peel puncture resistance (PPR) of 'Hamlin' oranges and time between application of GA_3 and wash-off spray, 29 Nov. 1999. Fruit were treated on 7 Oct. 1998. Each point represents the mean of a 10-fruit sample selected from a single tree. Model $y = 5.87 + 1.13x$, join-point = 1.39, $r^2 = 0.35$.

were treated with GA_3 but not washed-off had slightly higher peel hue angles and PPR at each harvest than trees receiving any other treatment (data not shown), which indicates that wash-off sprays reduced GA_3 efficacy. Thus, it is likely that in 1998-99, most GA_3 was absorbed within 1 h of application.

Wash-off treatments had a greater effect on peel hue angle and PPR in 1999-2000 than they did the previous season (Figs. 1-3 and 1-4). Peel hue angle data had a smaller initial slope and a later join-point (1.78 h) on 29 Nov. than on 8 Jan. 1999. Peel puncture resistance data also had a later join-point (1.4 h) on 29 Nov. than 8 Jan. 1999. The rate of GA_3 uptake was more rapid in 1998 than in 1999, possibly due to differences in temperature. The permeability of citrus cuticles rapidly increases with temperature (Baur et al., 1997) and the average temperature during application was about 2 °C higher in 1998 than 1999 (25 °C vs. 23 °C, respectively).

As in 1998, trees that were sprayed with GA_3 but not washed-off had higher peel hue angles and PPR than other treatments (data not shown), indicating that uptake persisted for hours or days after application. As in the previous season, peel hue angle data better fit a linear plateau model better than did PPR data (r^2= 0.68 vs. 0.35, respectively), so peel hue angle might be a more useful indicator of GA_3 efficacy than PPR.

Logistical problems limited the number of replicate trees and selection of wash-off times. The relatively high variability among trees and the low number of wash-off times (particularly between 0 and 2 h after application) probably weakened the regression model. Nevertheless, these data generally agree with laboratory studies that show high absorption of ^{14}C-GA_3 within 1 h of application followed by continued uptake for several

hours (Greenberg and Goldschmidt, 1990; Ferguson et al., 1986). Therefore, it is likely that rainfall 2 h after application or later will have little effect on GA_3 efficacy. However, the fact that non-washed fruit usually had the highest hue and PPR values (data not shown) indicates that GA_3 absorption continues at a slow rate for an indefinite time, as suggested by others (Ferguson et al., 1986).

Conclusions. Growers and researchers applying GA_3 to citrus should be aware that the initial 1-2 h period after application is most essential for uptake. Moreover, even longer periods without rainfall are desirable to insure maximal response, even when using a surfactant.

CHAPTER 4
GIBBERELLIC ACID APPLICATION TIMING AFFECTS FRUIT QUALITY OF PROCESSING ORANGES

Introduction

Gibberellic acid (GA_3) has been applied to citrus trees since the late 1950s to delay peel maturity and thereby prevent, or reduce the severity of, senescence-related peel disorders (Coggins, 1969; Davies, 1986). In the fall, cool temperatures induce peel color break, a green to yellow color change of the flavedo as chloroplasts are converted to chromoplasts (Coggins and Jones, 1977) and peel puncture resistance (PPR) gradually declines (Coggins and Lewis, 1965). Peel senescence may become pronounced, especially if fruit is "stored" on the tree for several months after attaining legal maturity. Tree-stored fruit can retain good internal quality for several months; however, the peel of non-GA_3 treated fruit may deteriorate because peel senescence sometimes begins before fruit has attained internal maturity (Coggins, 1969). Senescent peels are typically thought of as a fresh fruit problem (Davies, 1986), but fruit that are mechanically damaged during harvest or those with overly soft or deformed rinds are also culled before processing (Davies et al., 1997).

The potential for GA_3 to extend the fresh citrus fruit harvest season recently prompted Florida citrus growers and processors to test the effects of GA_3 on processing fruit (Davies et al., 1997). Early reports suggested that GA_3 did not affect juice yield (Coggins, 1969; Davies, 1986), but recent research found that GA_3-treated fruit yielded up to 10% more juice than non-treated fruit (Davies et al., 1997; Davies et al., 1999;

Davies et al., 2001). Such an increase would be of considerable economic benefit to Florida citrus growers because processed fruit value increases with juice yield and Brix (Braddock, 1999). Therefore it is desirable to develop management practices for optimization of juice yield by GA_3.

The efficacy of GA_3 with respect to peel senescence is related to the physiological age of the fruit and to the amount of time between application and harvest (Greenberg et al., 1992). Thus, timing of GA_3 application might also be critical for maximization of juice yield (Davies et al., 1999). Late fall application of GA_3 can reduce or delay subsequent flowering in citrus (Monselise and Halevy, 1964); a factor that must be considered in timing recommendations. Thus, research was conducted between 1998 and 2000 to determine the effect of GA_3 application timing on fruit quality and subsequent flowering of 'Hamlin', 'Pineapple' and 'Valencia' sweet oranges in Florida.

Materials and Methods

Plant material. Experiments were conducted on 'Hamlin', 'Pineapple' and 'Valencia' orange [*Citrus sinensis* (L.) Osb.] trees in 1998 and 1999. Trees were on Bittersweet (*Citrus aurantium* L.), sour orange (*Citrus aurantium* L.) or Carrizo citrange [*Citrus sinensis* (L.) Osb. x *Poncirus trifoliata* (L.) Raf.] rootstocks, respectively. 'Hamlin' and 'Pineapple' trees were planted in 1981 and 'Valencia' trees in 1975. All trees were located in a commercial orchard near Arcadia, Fla. Experimental trees were selected on the basis of uniform size, appearance and estimated crop load and were surrounded by non-experimental border trees.

Application of GA_3. A back-pack sprayer (Solo, Newport News, Va.) was used to apply approximately 10 L of a GA_3 (ProGibb®, Valent BioSciences, Chicago, Ill.; 45

g a.i./ha) and organo-silicone surfactant (Silwet®, Setre Chemical Co., Memphis, Tenn.; 0.05% v/v) solution to each tree. Applications were made on 12 different mature 'Hamlin' and 'Pineapple' orange trees on 2 Sept., 25 Sept., 10 Oct. (before color break; hue angle ≥ 120°) or 12 Nov. 1998 (after color break; hue angle ca. 107°) and 25 Sept. (before color break; hue angle ≥ 120°), 27 Oct. (about color break; hue angle ca. 110°), or 19 Nov. 1999 (after color break; hue angle ca. 100°). The same solution was also applied to 12 different 'Valencia' orange trees on 25 Sept., 10 Oct., 12 Nov. (before color break; hue angle > 120°) or 8 Dec. 1998 (after color break; hue angle ca. 108°) and 25 Sept., 27 Oct. (before color break; hue angle ≥ 120°), 19 Nov. (about color break; hue angle ca. 110°) or 9 Dec. 1999 (after color break; hue angle ca. 105°). Each year, 12 additional 'Hamlin', 'Pineapple' and 'Valencia' trees remained non-sprayed (controls). The experiment consisted of a randomized complete block design with five treatments and 12 single tree replications.

Each tree received the same treatment both years except for 1999 when 'Hamlin' orange trees did not receive an early September spray and 'Pineapple' orange trees were not tested after the grower applied GA_3 to the entire 'Pineapple' grove. Application timing and the number of replicates used were suggested by previous studies (Davies et al., 1997; Davies et al., 1999).

Fruit quality tests. Fruit were harvested two or three times each season during the normal harvest period of each cultivar. At each harvest, two samples of fruit were randomly collected at a 1- to 2m height around each tree. One sample consisted of 10 fruit that were washed, air dried, and evaluated for peel color, peel puncture resistance (PPR), fruit diameter and peel thickness. Peel color (hue) was measured with a Minolta

chroma meter (Minolta Inc., Ramsey, N.J.). Peel puncture resistance was measured with an AccuForce® force gauge (Ametek, Largo, Fla.) that recorded the peak force sustained by the peel when subjected to a cylindrical, 0.5 mm diameter, steel probe. Fruit diameter and peel thickness were measured at the fruit equator with calipers. Average peel color and PPR of each fruit were determined from three measurements per fruit (made equidistant around the fruit equator). Additional samples of about 10 kg of fruit were collected from each tree and transported to the Department of Citrus at Lake Alfred, Fla. for juice yield and quality tests. Each sample was juiced according to Florida State test standards (Wardowski et al., 1995) using an FMC Model 091 juice extractor. Juice weight, Brix, acid and ratio were determined using standard techniques (Wardowski et al., 1995).

Flowering. On 1 Apr. 1999, all trees were inspected and rated according to their stage of flowering. A scale of 1 to 10 was used to classify the stage of flowering with 1 = no open flowers, 2 = 25% open flowers, 3 = 50% open flowers, 4 = 75% open flowers, 5 = full bloom, 6 = 25% petal fall, 7 = 50% petal fall, 8 = 75% petal fall, 9 = 100% petal fall, 10 = initial fruit set. Flowering was not evaluated in 2000.

Experimental design and analysis. The experiment was established as a randomized complete block design, but there were no block effects (data not shown), so the data were re-analyzed as a completely randomized design. Data were subjected to the general linear model procedure (SAS Institute Inc., Cary, NC, USA) for analysis of variance (ANOVA). To test whether the variables changed over time, data were subjected to ANOVA as a split-plot in time with treatments as the main plot and harvest time as the subplot. The effect of harvest time was significant for each variable ($P \leq$

0.05; data not shown) and data were then subjected to analysis of variance by date and treatment. When treatment effects were significant, treatment means were separated within dates using Duncan's New Multiple Range Test (DNMRT). To examine relationships among variables, fruit quality data were subjected to regression analysis using PROC REG (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Peel hue angle (H°) and PPR decreased with time for each cultivar during both seasons (Tables 1-5). Fruit from GA_3-treated trees usually had higher H° (were more green) and greater PPR than fruit of control trees; however, application timing affected both variables. For example, peel H° of 'Hamlin' and 'Pineapple' fruit treated with GA_3 in early September was often comparable to that of non-sprayed fruit by the final harvest (Tables 1-3), whereas fruit receiving late sprays generally had greener peels than other fruit, particularly for late harvests. In contrast, the highest PPR was usually achieved with an early GA_3 application, even for late harvests (Tables 4-1 to 4-5).

Thus peel color development may be inhibited or reversed (Huff, 1984), even by post-color break sprays, but the progressive decrease in PPR can best be slowed by GA_3 applied before color break. Moreover, the GA_3 effect on PPR is longer lasting than its effect on peel color (if applied early enough). These findings are consistent with the effects of GA_3 on puffing of mandarin and creasing of orange where early application retards the breakdown of albedo tissue without affecting peel color development (Greenberg et al., 1992; Monselise et al., 1976; Pozo et al., 2000). Therefore, GA_3

Table 4-1. Peel color (hue angle) and peel puncture resistance (PPR) of 'Hamlin' oranges sprayed with GA_3 (45 g a.i./ha) on different dates, Arcadia, Fla., 1998-99.

GA_3 application date, 1998	Harvest date					
	8 Dec. 1998	20 Jan. 1999	17 Feb. 1999	8 Dec. 1998	20 Jan. 1999	17 Feb. 1999
	Hue angle (°)			PPR (N)		
2 Sept.	106.4 c[z]	85.8 d	81.7 bc	5.4 b	3.8 c	3.9 a
25 Sept.	114.2 a	92.7 c	85.8 b	6.0 a	4.4 ab	4.0 a
10 Oct.	112.1 ab	95.5 b	85.6 b	5.8 a	4.5 a	4.0 a
12 Nov.	109.8 b	101.9 a	90.9 a	5.2 b	4.2 b	4.0 a
Non-sprayed	99.2 d	82.8 e	80.8 c	4.5 c	3.4 d	3.6 b

[z]Means followed by a different letter are significantly different within columns by Duncan's New Multiple Range Test, $P \leq 0.05$. Ten fruit were collected from each of 12 trees/treatment. Three measurements were made equidistant around the fruit equator.

Table 4-2. Peel color (hue angle) and peel puncture resistance (PPR) of 'Hamlin' oranges sprayed with GA_3 (45 g a.i./ha) on different dates, Arcadia, Fla., 1999-00.

	Harvest date			
GA_3 application	7 Dec. 1999	5 Jan. 2000	7 Dec. 1999	5 Jan. 2000
date, 1999	Hue angle (°)		PPR (N)	
25 Sept.	104.7 a[z]	90.1 b	6.1 a	4.9 a
27 Oct.	103.9 a	93.2 a	5.3 b	4.7 a
19 Nov.	95.8 b	89.8 b	4.7 c	4.4 b
Non-sprayed	85.6 c	77.4 c	4.0 d	3.5 c

[z]Means followed by a different letter are significantly different within columns by Duncan's New Multiple Range Test, $P \leq 0.05$. Ten fruit were collected from each of 12 trees/treatment. Three measurements were made equidistant around the fruit equator.

Table 4-3. Peel color (hue angle) and peel puncture resistance (PPR) of 'Pineapple' oranges sprayed with GA_3 (45 g a.i./ha) on different dates, Arcadia, Fla., 1998-99.

GA_3 application date, 1998	Harvest date, 1999			
	3 Feb.	24 Feb.	3 Feb.	24 Feb.
	Hue angle (°)		PPR (N)	
2 Sept.	84.8 b^z	79.4 b	5.2 a	5.1 a
25 Sept.	82.3 b	77.4 b	4.0 b	3.9 b
10 Oct.	96.6 a	89.0 a	5.2 a	5.0 a
12 Nov.	95.3 a	91.1 a	4.7 ab	4.5 ab
Non-sprayed	78.9 b	74.7 b	4.0 b	3.9 b

^{z}Means followed by a different letter are significantly different within columns by Duncan's New Multiple Range Test, $P \leq 0.05$. Ten fruit were collected from each of 12 trees/treatment. Three measurements were made equidistant around the fruit equator.

Table 4-4. Peel color (hue angle) and peel puncture resistance (PPR) of 'Valencia' oranges sprayed with GA_3 (45 g a.i./ha) on different dates, Arcadia, Fla., 1998-99.

GA_3 application date, 1998	Harvest date, 1999			
	23 Apr.	13 May	23 Apr.	13 May
	Hue angle (°)		PPR (N)	
25 Sept.	91.0 bc[z]	91.8 b	7.0 a	6.4 a
10 Oct.	90.0 c	88.6 c	6.4 bc	5.8 b
12 Nov.	93.7 b	93.8 ab	6.5 b	6.0 b
8 Dec.	97.5 a	95.4 a	6.6 b	6.1 ab
Non-sprayed	85.4 d	85.7 d	6.1 c	5.6 b

[z]Means followed by a different letter are significantly different within columns by Duncan's New Multiple Range Test, $P \leq 0.05$. Ten fruit were collected from each of 12 trees/treatment. Three measurements were made equidistant around the fruit equator.

Table 4-5. Peel color (hue angle) and peel puncture resistance (PPR) of 'Valencia' oranges sprayed with GA_3 (45 g a.i./ha) on different dates, Arcadia, Fla., 2000.

GA_3 application date, 1999	Harvest date					
	10 Feb.	10 Mar.	10 May	10 Feb.	10 Mar.	10 May
	Hue angle (°)			PPR (N)		
25 Sept.	81.4 c^z	80.0 c	79.9 b	5.3 c	5.0 d	5.1 c
27 Oct.	104.8 a	98.2 a	93.7 a	7.4 a	7.4 a	6.5 a
19 Nov.	102.5 a	97.9 a	93.0 a	6.1 b	6.2 b	5.8 b
9 Dec.	98.7 b	93.6 b	92.3 a	5.8 b	5.7 c	5.8 b
Non-sprayed	81.1 c	80.0 c	79.0 b	5.3 c	5.3 d	5.3 c

[z]Means followed by a different letter within columns are significantly different within columns by Duncan's New Multiple Range Test, $P \leq 0.05$. Ten fruit were collected from each of 12 trees/treatment. Three measurements were made equidistant around the fruit equator.

effects on peel hue and PPR, though often positively correlated (Coggins and Lewis, 1965), appear to be independent. Gibberellic acid effects on peel senescence were less persistent for 'Hamlin' or 'Pineapple' (Tables 4-1 to 4-3) than for 'Valencia' (Tables 4-5); about 5-6 months vs. 7 to 8 months, respectively.

Juice yield remained relatively constant among harvest dates for each cultivar (Tables 4-6, 4-7). However, there were significant treatment effects on juice yield of 'Hamlin' and 'Valencia' oranges on certain harvests during both seasons. 'Hamlin' oranges sprayed on 25 Sept. 1998 yielded more juice than non-sprayed fruit on 8 Dec. 1998. On 20 Jan. 1999, fruit sprayed on 10 Oct. 1998 yielded more juice than fruit treated on 2 Sept. 1998 but not more than non-sprayed fruit (Table 4-6). There were no treatment effects on juice yield on 17 Feb. 1999. Thus, the greatest juice yield for 'Hamlin' oranges was achieved when fruit were harvested between 1.5 to 3 months after application. The following season, there were no significant treatment effects on 7 Dec. 1999. However, on 5 Jan. 2000, fruit treated on 27 Oct. 1999 (about 2 months before harvest) yielded more juice than non-sprayed fruit or fruit treated on 12 Nov. 1999.

Gibberellic acid application on 12 Nov. 1998 increased 'Valencia' juice yield over non-sprayed fruit when harvested on 23 Apr. 1999, about 5 months later (Table 4-7), but treatments did not affect juice yield on 13 May 1999. The following season, 'Valencia' fruit sprayed on 27 Oct. or 19 Nov. 1999 yielded more juice on 10 Mar. 2000 than fruit sprayed on 25 Sept. or non-sprayed fruit (Table 4-7). On 10 May 2000, fruit sprayed on 9 Dec. yielded more juice than non-sprayed fruit, though fruit sprayed on 25 Sept. yielded less juice than other GA_3 application times and non-sprayed fruit. Thus, GA_3 increased juice yield of 'Valencia' oranges in 1998-99 and 1999-2000 when applied

Table 4-6. Juice yield of 'Hamlin' oranges sprayed with GA_3 (45 g a.i./ha) on different dates, Arcadia, Fla., 1998-99, 1999-2000.

1998-99 Season				1999-2000 Season		
GA_3 application date, 1998	Harvest date, 1998-99			GA_3 application date, 1999	Harvest date, 1999-2000	
	8 Dec.	20 Jan.	17 Feb.		7 Dec.	5 Jan.
	Juice yield (%wt/wt)				Juice yield (%wt/wt)	
2 Sept.	59.4 b[z]	58.9 b	59.5 a	-	-	-
25 Sept.	62.1 a	60.6 ab	60.4 a	25 Sept.	59.1 a	58.5 ab
10 Oct.	60.8 ab	61.5 a	60.3 a	27 Oct.	59.9 a	59.1 a
12 Nov.	61.0 ab	59.3 ab	60.1 a	19 Nov.	58.0 a	57.3 b
Non-sprayed	59.5 b	60.4 ab	59.3 a	Non-sprayed	58.6 a	57.5 b

[z]Means followed by a different letter are significantly different within columns by Duncan's New Multiple Range Test, $P \leq 0.05$. Means of a 10-kg fruit sample/tree from 12 trees/treatment.

Table 4-7. Juice yield of 'Valencia' oranges sprayed with GA_3 (45 g a.i./ha) on different dates, Arcadia, Fla., 1998-99, 1999-2000.

1998-99 Season			1999-2000 Season			
GA_3 application date, 1998	Harvest date, 1999		GA_3 application date, 1999	Harvest date, 2000		
	23 Apr.	13 May		10 Feb.	10 Mar.	10 May
	Juice yield (%wt/wt)			Juice yield (%wt/wt)		
25 Sept.	61.0 ab[z]	60.2 a	25 Sept.	58.9 a	58.0 c	60.2 c
10 Oct.	60.8 ab	60.3 a	27 Oct.	58.3 a	61.9 a	61.7 ab
12 Nov.	62.4 a	61.2 a	19 Nov.	61.0 a	62.0 a	61.8 ab
8 Dec.	61.9 ab	60.0 a	9 Dec.	60.4 a	60.8 ab	62.1 a
Non-sprayed	60.4 b	60.6 a	Non-sprayed	60.8 a	59.1 bc	60.4 bc

[z]Means followed by a different letter are significantly different within columns by Duncan's New Multiple Range Test, $P \leq 0.05$. Means of a 10-kg fruit sample/tree from 12 trees/treatment.

about 5 months before harvest. Juice yield of 'Pineapple' oranges was not affected by GA_3 application during the one season tested (data not shown).

The magnitude of the juice yield increase (about 4%, standardized to control) was comparable to that observed in previous studies (Davies et al., 1997; Davies et al., 1999; Davies et al., 2001). Time between application and harvest affected the amount of juice increase because the optimal application time advanced with harvest date for 'Hamlin' in 1998-99 and for 'Valencia' during both seasons. Juice yield of 'Hamlin' responded to a shorter time between application and harvest than did 'Valencia', which was also observed for peel color and PPR. Although GA_3 did not enhance juice yield of 'Hamlin' nearly as frequently as it did for 'Valencia' (relative to controls, only the 25 Sept. 1998 application increased juice yield of 'Hamlin' on 8 Dec. 1998), these findings agree with those of Davies et al. (1997) who observed that GA_3 applied in mid-September to two different groves increased juice yield of 'Hamlin' in both groves in mid-December but not in January or February. Apparently, application date is more critical for 'Hamlin' than for 'Valencia'. The fact that GA_3 did not affect juice yield of 'Pineapple' is surprising because GA_3 substantially increased juice yield of this cultivar in a previous study (Davies et al., 1997). However, GA_3 was less effective in modifying fruit quality variables of 'Pineapple' fruit compared with 'Hamlin' or 'Valencia'.

How GA_3 increased orange juice yield is unknown. Autumn application of GA_3 increased late-season juice yield of satsuma mandarin (*Citrus unshiu* Marc.) and 'Sunburst' tangerine [(*C. paradisi* Macf. x *C. reticulata* Blanco) x *C. reticulata* Blanco] (Garcia-Luis et al., 1985; Pozo et al., 2000) by suppressing the normal decrease in juice content. However, in this study GA_3 increased juice yield of 'Hamlin' and 'Valencia'

relatively early in the season when juice yield was not appreciably declining. Moreover, GA_3-treated 'Hamlin' and 'Valencia' fruit always had the highest juice yield of the season.

Gibberellic acid reduced the peel:pulp ratio of mandarins (Garcia-Luis et al., 1985; Pozo et al., 2000) but in the present study, neither fruit diameter nor peel thickness was affected by GA_3 (data not shown). Therefore, GA_3 probably did not increase juice yield by increasing pulp volume. Differential water loss between GA_3 and non-GA_3 treated fruit probably cannot account for differential juice yield because GA_3 did not affect peel wax content (El-Otmani et al., 1986) or fruit water loss (El-Otmani and Coggins, 1991), though it did affect wax composition (El-Otmani et al., 1986).

An alternative hypothesis is that GA_3 altered the physical or rheological properties of the fruit such that the efficiency of mechanical juice extraction was enhanced. Oranges with senescent peels sometimes burst during juice extraction (J. Keithly, pers. com.), possibly allowing some pulp to escape the juice stream. Thus, fruit having GA_3-strengthened peels might lose less juice during extraction than non-sprayed fruit having mechanically weaker peels. In an earlier study, PPR was highly correlated with juice yield (Davies et al., 1997), providing further evidence that GA_3 might enhance juice yield by increasing peel strength. However, in the present study, there was no consistent correlation between PPR and juice yield. Nevertheless, a study was conducted to determine whether GA_3 affects various fruit rheological properties that might affect juice yield (Chapter 5).

Table 4-8. Juice Brix of 'Hamlin' oranges sprayed with GA_3 (45 g a.i./ha) on different dates, Arcadia, Fla., 1998-99, 1999-2000.

1998-99 Season				1999-2000 Season		
GA_3 application date, 1998	Harvest date			GA_3 application date, 1999	Harvest date	
	8 Dec.	20 Jan.	17 Feb.		7 Dec. 1999	5 Jan. 2000
	Juice Brix (°)				Juice Brix (°)	
2 Sept.	10.0 ab[z]	11.2 a	12.0 a	-	-	-
25 Sept.	9.8 bc	11.0 ab	12.0 a	25 Sept.	9.9 b	10.6 b
10 Oct.	9.7 c	10.8 bc	11.6 ab	27 Oct.	10.2 ab	10.7 ab
12 Nov.	9.7 c	10.6 c	11.4 b	19 Nov.	10.3 a	10.6 b
Non-sprayed	10.1 a	11.2 a	11.8 a	Non-sprayed	10.4 a	11.0 a

[z]Means followed by a different letter are significantly different within columns by Duncan's New Multiple Range Test, $P \leq 0.05$. Means of a 4 L/tree juice sample from 12 trees/treatment.

Table 4-9. Juice Brix of 'Valencia' oranges sprayed with GA_3 (45 g a.i./ha) on different dates, Arcadia, Fla., 1998-99, 1999-00.

1998-99 Season			1999-2000 Season			
GA_3 application date, 1998	Harvest date, 1999		GA_3 application date, 1999	Harvest date, 2000		
	23 Apr.	13 May		10 Feb.	10 Mar.	10 May
	Juice Brix (°)			Juice Brix (°)		
25 Sept.	12.6 b[z]	12.8 b	25 Sept.	12.1 a	13.0 a	13.6 a
10 Oct.	12.6 b	13.0 ab	27 Oct.	11.4 b	12.2 d	12.8 c
12 Nov.	12.5 b	12.7 bc	19 Nov.	11.6 b	12.4 cd	13.1 bc
8 Dec.	12.4 b	12.4 c	9 Dec.	11.6 b	12.6 bc	12.8 c
Non-sprayed	13.3 a	13.3 a	Non-sprayed	12.1 a	12.9 ab	13.4 ab

[z]Means followed by a different letter are significantly different within columns by Duncan's New Multiple Range Test, $P \leq 0.05$. Means of a 4 L/tree juice sample from 12 trees/treatment.

Juice Brix increased during the harvest season for all cultivars as expected. However, some GA_3 treatments resulted in lower juice Brix at harvest during both seasons for all cultivars (Tables 4-8, 4-9). 'Hamlin' oranges treated with GA_3 on 25 Sept., 10 Oct. and 12 Nov. 1998 had lower juice Brix than control fruit on 8 Dec. 1998. On 20 Jan. 1999 the 10 Oct. and 12 Nov. 1998 GA_3 treatments reduced juice Brix, but only the 12 Nov. 1998 application resulted in lower Brix by 17 Feb. 1999. Thus, GA_3 reduced 'Hamlin' juice Brix within 1 month of application and the effect persisted for 2.5 to 3 months. In 1999-2000, only GA_3 applied about 2 months before harvest reduced juice Brix on 7 Dec., but GA_3 applied on all dates reduced juice Brix on 5 Jan. which was between 2 and 3 months after application.

Effects of GA_3 on 'Valencia' juice Brix lasted longer than for 'Hamlin'. All GA_3 treatments resulted in lower 'Valencia' juice Brix than controls for fruit harvested on 23 Apr. and 13 May 1999, except for fruit treated on 27 Oct. 1998 that did not have lower juice Brix than control fruit on 13 May. Therefore, GA_3 effects persisted up to 7 months. Similar results were observed in 2000 when GA_3 applied between 27 Oct. and 9 Dec. 1999 generally reduced juice Brix from 10 Feb. through 10 May. As observed for other fruit quality variables measured, 'Pineapple' juice Brix was much less affected by GA_3 than was 'Hamlin' or 'Valencia', with only the 12 Nov. 1998 application date reducing juice Brix on 3 Feb. 1999 (data not shown).

Most studies indicate that GA_3 does not affect juice Brix (Coggins, 1969; Davies, 1986; Davies et al., 1997). However, Coggins et al. (1960b) found that green peel color increased and juice Brix decreased related to the amount of GA_3 applied to 'Valencia' oranges. Cary (1975) also reported that gibberellin sprays accentuated regreening and

reduced juice Brix of 'Valencia' oranges when both mature and small fruit were on the tree. Sites and Reitz (1950) found that 'Valencia' oranges with green peels tended to have lower total soluble solids than fruit with yellow or orange colored peels. Similarly, Pozo et al. (2000) reported that GA_3 sprays temporarily reduced 'Sunburst' tangerine juice Brix. Thus, these findings add to the evidence that fruit having natural or GA_3-induced green peel color may also have lower juice Brix.

It is not known how GA_3 could reduce juice Brix. Brix was not related to juice yield ($r^2 \cong 0$), so the reduction in Brix did not occur by dilution. However, there was an inverse association between green peel color and juice Brix. Application dates that resulted in the highest peel hue angles (most green color) usually had the lowest juice Brix, whereas control fruit, which usually had the lowest peel hue angles, typically had the highest juice Brix. Peel hexose levels are inversely related to peel chlorophyll levels (Huff, 1984) and GA_3 increased navel orange peel respiration (Lewis et al., 1967). Perhaps GA_3 affects the peel vs. the pulp sink strength. The relationship between GA_3 and juice Brix is discussed in Chapter 7.

The value of fruit grown for processing in Florida is based on the weight of solids (°Brix x juice weight) per weight of whole fruit (Wardowski et al., 1995). In this study, treatments sometimes reduced yield of solids if they did not increase juice yield but did reduce juice Brix (data not shown). None of the treatments increased yield of solids compared to non-sprayed fruit.

Applications of GA_3 in 1998 did not delay flowering of 'Hamlin' orange in 1999 (Table 10). In contrast, all GA_3 applications delayed flowering of 'Pineapple', and late September and mid-November sprays delayed flowering of 'Valencia' trees. Gibberellic

Table 4-10. Flower rating of 'Hamlin', 'Pineapple' and 'Valencia' orange trees, 1 Apr. 1999.

GA_3 application date, 1998	Flower rating[z]		
	Hamlin	Pineapple	Valencia
2 Sept.	7.5 a[y]	2.7 b	-
25 Sept.	8.8 a	3.3 b	3.8 b
27 Oct.	9.0 a	2.8 b	5.0 ab
19 Nov.	7.8 a	2.6 b	2.9 b
9 Dec.	-	-	4.3 ab
Non-sprayed	8.7 a	6.5 a	6.8 a

[z]A scale of 1 to 10 was used to classify the stage of flowering with 1 = no open flowers, 2 = 25% open flowers, 3 = 50% open flowers, 4 = 75% open flowers, 5 = full bloom, 6 = 25% petal fall, 7 = 50% petal fall, 8 = 75% petal fall, 9 = 100% petal fall, 10 = initial fruit set.

[y]Means followed by a different letter are significantly different within columns by Duncan's New Multiple Range Test, $P \leq 0.05$. Values are means of 12 trees/treatment.

acid applied between November and January may inhibit citrus flower induction (Monselise and Halevy, 1964) as may the presence of fruit (Garcia-Luis et al., 1986). Thus, the cultivar's differential response to GA_3 might be related to their harvest time. In 1999, 'Hamlin' fruit were harvested in February but 'Pineapple' fruit were harvested in March (a few weeks prior to anthesis) and 'Valencia' fruit were not harvested until May (post-anthesis). Garcia-Luis et al. (1986) contend that the fruit of a parthenocarpic cultivar of Satsuma mandarin (*Citrus unshiu* Marc.) only inhibit flowering until they have completed peel color development. This does not appear to be the case in the present study, however, because all GA_3 treatments delayed peel color development of 'Hamlin' through 17 Feb. but only two GA_3 treatments delayed peel color development of 'Pineapple' by that date.

In conclusion, GA_3 applied at about color break can enhance juice yield of 'Hamlin' and 'Valencia' oranges that are harvested 2 to 5 months later. Treatment effects on juice yield were variable but effective application and harvest dates were consistent with previous studies (Davies et al., 1997; Davies et al., 1999; Davies et al., 2001). However, GA_3 treatments also resulted in lower juice Brix in some cases, and yield of solids was sometimes reduced if treatments that reduced juice Brix did not increase juice yield. Additional research is needed to determine whether the positive effects of GA_3-treatment on juice yield may be separated from negative effects on juice Brix. Treatments delayed flowering of 'Pineapple' and 'Valencia' but not 'Hamlin', possibly because 'Hamlin' fruit were harvested earlier than the other cultivars.

CHAPTER 5
MECHANICAL PROPERTIES OF ORANGE PEEL AND FRUIT TREATED PREHARVEST WITH GIBBERELLIC ACID

Introduction

Basic mechanical property data of citrus peel and fruit are limited, despite the fruit's economic importance. Citrus fruit are a unique type of berry called a hesperidium (Schneider, 1969). Their leathery peel is composed of two anatomically distinct layers known as the flavedo and albedo. The flavedo, the outer, colored-portion of the peel, consists of a cuticle-covered epidermis and a few layers of tabular, thick-walled cells interspersed with oil glands (Albrigo and Carter, 1977). The albedo, the white, inner portion of the peel, consists of several layers of branched, thin-walled cells, numerous intercellular air spaces, and a few oil glands. Albedo thickness may vary widely among citrus species and cultivars but, except for certain mandarin cultivars, the albedo typically accounts for most of the peel thickness (Spiegel-Roy and Goldschmidt, 1996). The peel surrounds 10-14 ovarian locules ('segments') that are arranged around an axis of spongy white tissue that is anatomically similar to the albedo.

The complex structure of citrus fruit might partly explain why mechanical property data are limited and why most mechanical property data are derived from pieces of citrus peel or flesh, instead of whole fruit. For example, Ahmed et al. (1973) reported shear stress data of peel pieces collected on one harvest date. Likewise, Kaufmann (1970) measured the effect of water potential and temperature on the extensibility of citrus peel. Gyasi et al. (1981) determined Poisson's ratio for citrus fruit peel and pulp

but did not publish the moduli used in calculations. Churchill et al. (1980) conducted rupture and tensile tests on peel pieces and whole fruit, but only reported maximum force and deformation data. In contrast, Miller (1986) determined stress index, modulus of elasticity and rupture force of freeze-damaged and non-damaged fruit. Sarig and Nahir (1973) reported the initial and permanent deformations of a creep test of citrus fruit to indicate firmness.

Citrus are treated with gibberellic acid (GA_3), a plant-growth regulator, in the late summer or fall to improve peel integrity and thus extend the fresh-fruit harvest season (Davies, 1986). The peel of GA_3-treated fruit retains a green color and is more resistant to puncture than the peel of non-treated fruit for several months after application (Coggins, 1969; Coggins and Lewis, 1965). There are many reports that GA_3-treatment improves 'firmness' of citrus fruits (Coggins, 1965; Davies, 1986), but horticulturists generally consider fruit puncture resistance and firmness as synonyms (Harker et al., 1997). Because the peel of fruit treated with GA_3 is more resistant to puncture than non-treated fruit, this treatment might make fruit less susceptible to mechanical damage (Davies et al., 1997). However, the effects of GA_3-treatment on basic mechanical properties of citrus peel or fruit have not been reported.

Recently it was suggested that improved juice yield of GA_3-treated oranges might be related to treatment effects on mechanical properties of the peel or fruit (Davies et al., 1997; Fidelibus et al., 2002b; J. Keithly, pers. com.). Most juice processors in Florida use extraction machines that squeeze fruit between two inter-digitating cups (Braddock, 1999). Cutters in each cup punch holes in the fruit that allow the peel to be extruded through the top cup while the pulp enters the juice stream through the bottom cup. Thus,

the extraction subjects the peel or fruit or both to compression, shear and tensile forces. It had been postulated that GA_3-treatment effects on mechanical properties related to these forces might enhance juice extraction efficiency. This hypothesis was tested on 'Valencia' oranges, but none of the variables measured correlated well with juice yield ($r^2 \leq 0.18$). Therefore, the objective of this paper is to report basic mechanical properties of GA_3-treated and non-treated orange peel and fruit that may be of value for growers, handlers and processors of citrus fruit.

Materials and Methods

Treatment Applications

About 10 L of a GA_3 (ProGibb®, Valent USA Corp., Libertyville, Ill.; 45 g a.i./ha) and organo-silicone surfactant (Silwet®, Helena Chemical Co., Memphis, Tenn.; 0.05% v/v) solution was applied with a back-pack sprayer (Solo, Newport News, VA) to 11 different mature 'Valencia' orange [*Citrus sinensis* (L.) Osb.] trees on 25 Sept., 12 Nov., or 8 Dec. 1998. The dates selected were before, near and after color break (the change in peel color from green to yellow), respectively. Eleven additional trees remained non-sprayed (controls). Sprays were applied between 0700 and 1000 HR. To prevent drift, each experimental tree was surrounded with non-experimental trees. All trees were located in a commercial grove near Arcadia, Fla. On 23 Apr. (mid-season) and 13 May 1999 (late-season), 10 fruit were randomly collected at a 1- to 2-m height around each tree. The fruit were placed in plastic bags and stored at 4°C and about 95% relative humidity until measurements were made, for as long as 4 weeks.

Peel Shear Test

Peel pieces were carefully dissected from the equator of five randomly selected fruit/tree (12 trees/treatment). Immediately after removal, peel thickness was measured with calipers, and peel pieces of about 0.0025 m^2 were placed in a punch shear test cell consisting of two 0.02 m thick aluminum plates. Each plate had a 0.026 m diameter hole in the center. Steel pins held the plates in place. A cylindrical knife-edged cutter 0.0254 m in diameter attached to the load cell of a universal testing machine (Instron, Canton, Mass.) was used to shear the peel pieces. The load cell was rated for a maximum of 50 kN and the crosshead traveled at a speed of 0.00016 m s^{-1} (10 mm • min^{-1}). Rupture force was measured. As the cutter applied force to each peel, there was some settling and compaction of the peels (strain range of 0 to about 0.5; Fig. 5-1) before they began to shear. Shearing generally occurred between a strain range of about 0.5 to 1.0, and shear modulus (G) was calculated as the slope of these linear sections of the shear stress vs. shear strain curves (fig. 1). Shear stress (τ_s) was calculated as:

$$\tau_s = F/A = F/(\pi dt) \qquad [1]$$

with F = force, A = area of perimeter edge engaged by cylindrical cutter, d = diameter of cutter and t = peel thickness. Shear strain (γ) was calculated as:

$$\gamma = D/t \qquad [2]$$

where D = deformation. Shear strength (SS) was calculated as:

$$\tau_{st} = F_r/A \qquad [3]$$

where F_r = rupture force and A = area of perimeter edge as described above.

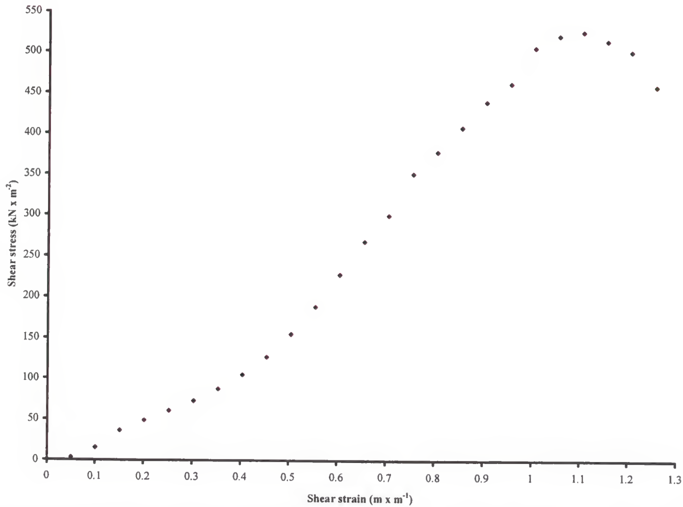

Fig. 5-1. Peel shear test. Typical stress vs. strain data for peel of 'Valencia' orange fruit. A cylindrical cutter (0.0254 m diam.) was attached to a 50 kN load cell travelling at a crosshead speed of 10 mm min^{-1}.

Peel Tensile Test

Peel pieces were carefully dissected from the equator of five randomly selected fruit/tree. Immediately after removal, peel thickness was measured with calipers, and peel strips of 0.02 m (polar) x 0.05 m (equatorial) were attached to Instron clips fitted with aluminum jigs. One clip was permanently attached to the base of the machine while the other was attached to a load cell rated at a maximum of 50 kN. Strips were subjected to axial loading in an equatorial direction, with a crosshead speed of 0.00016 m s^{-1} (10 mm min^{-1}) until rupture. Rupture force was measured. Modulus of elasticity (E_T) was calculated as the slope of the initial straight-line portion of a stress/strain curve (Fig. 5-2). In the sample chart, the peel began to yield after about 0.1 strain, and the remainder of the stress/strain curve was curvilinear. Tensile strength (σ_t) was calculated as:

$$\sigma_t = F_r/A \qquad [4]$$

where F_r = rupture force, and A = cross sectional area of peel strip (thickness x width).

Fruit Compression Test

Prior to fruit compression tests, the diameter and radius of curvature of each fruit was measured at the equator with a caliper and dial gauge, respectively. The fruits were supported by a cradle that consisted of a 0.075 m diameter (about the same as the fruit) hemispherical depression in the center of an aluminum plate. A flat stainless steel plate attached to a 50 kN load cell was positioned at the fruit equator and used to subject the fruit to a load/unload test at a rate of 0.00016 m s^{-1} (10 mm min^{-1}). Maximum displacement was 0.01 m. Degree of elasticity (D_e) was calculated as the ratio of elastic

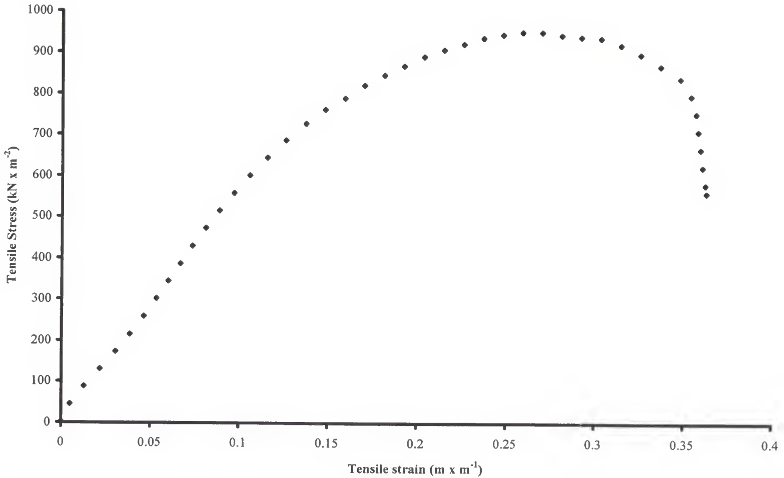

Fig. 5-2. Tensile test of 'Valencia' orange peel. Typical stress vs. strain data for peel of 'Valencia' orange fruit. Peel pieces were secured with clips that anchored one end of each peel strip to the base of the testing machine and the other end to a 50 kN load cell with a cross-head speed of 0.00016 m • s^{-1} (10 mm • min^{-1}).

to total deformation. Young's modulus (E) was calculated as:

$$E = [0.531\ (1-\mu^2)F']/(D^{3/2}) \bullet [(1/R_1) + (1/R_{1'})]^{1/2} \quad [5]$$

where $(1-\mu^2) = 0.89$ (Gyasi et al., 1981; Miller et al., 1986), F' = force, D = deformation, and R_1 and $R_{1'}$ are orthogonally intersecting radii of curvature at point of contact (ASAE, 1984). Generally, F' between D = 0.008 to 0.01 m was used to calculate E. Mechanical hysteresis was calculated as the area between the loading and unloading force vs. deformation curve and resilience was calculated as the area under the unloading force vs. deformation curve (shown in Fig. 5-3).

Fruit Cutting Test

Fruit used in compression tests were also subjected to a cutting test. Fruit cutting tests subjected whole fruit to the same cutter and load cell speed used in the peel shear tests with a cross head speed of 50 mm • min^{-1} (0.0008 m • s^{-1}). Each fruit was loaded with the knife-edged cutter positioned at the equator, opposite the point where fruit was loaded with a flat plate. Rupture force was measured and peel puncture strength was calculated by equation #3. Apparent modulus of elasticity (E_a) was calculated as the slope of the initial linear portion of the stress vs. strain curve (shown in Fig. 5-4) where stress (σ_c) was determined as:

$$\sigma_c = F/A = F/(\pi r^2) \quad [6]$$

where F = force, A = area of the circle inscribed by the cutter, and r = radius of the cutter. Strain (ε) was determined as:

$$\varepsilon = D/d \quad [7]$$

where D = deformation and d = fruit diameter.

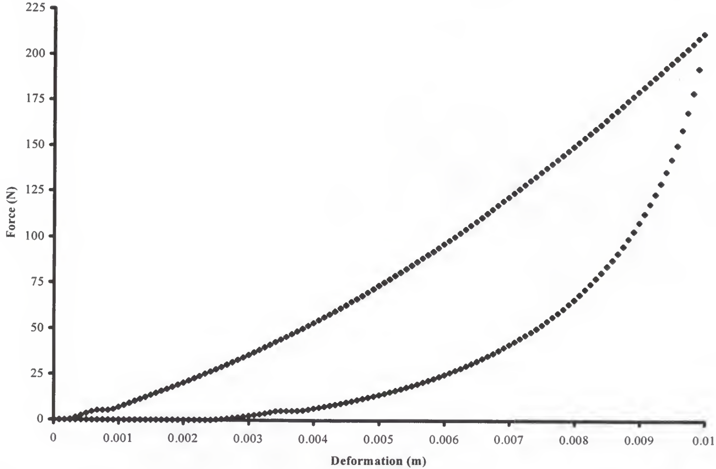

Fig. 5-3. Typical force vs. deformation data of whole 'Valencia' orange fruit subjected to axial loading and unloading with a flat plate. Fruit was supported by a hemispherical cradle with the fruit axis parallel to the plate. A 50 kN load cell was used and the crosshead speed was 0.00016 m • s^{-1} (10 mm • min^{-1}).

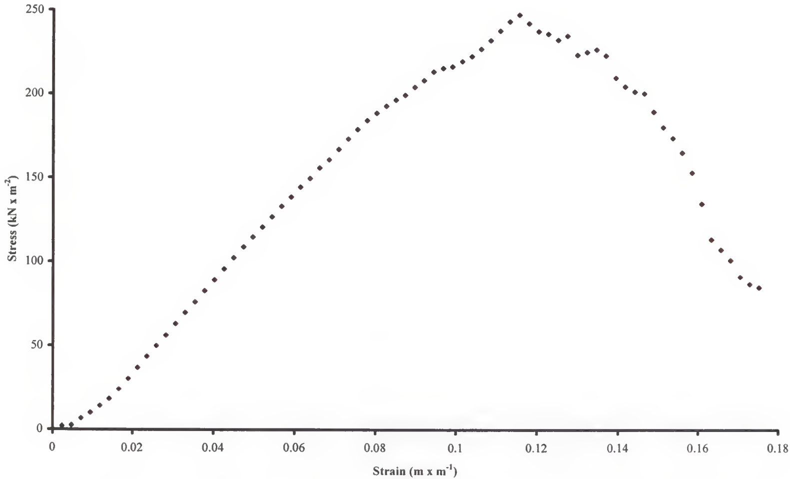

Fig. 5-4. Typical stress vs. strain data for whole ‘Valencia’ orange fruit subjected to axial loading with a 0.0254 m diameter cylindrical cutter. A hemispherical cradle supported fruit. A 50 kN load cell was used and the crosshead speed was 0.0008 m • s^{-1} (50 mm • min^{-1}).

Statistical Analysis

For each property measured, on each harvest date, the average of five fruit per tree was calculated and data of eleven trees per treatment were subjected to analysis of variance (PROC GLM; SAS Institute, Cary, NC). When treatment effects were significant ($P \leq 0.05$), treatment means were separated within dates by Duncan's New Multiple Range Test. Relationships between selected variables were tested by regression analyses (PROC REG; SAS Institute, Cary, NC).

Results and Discussion

Peel Shear Test

Peel rupture force, shear strength (τ_{st}), and G of treated and non-treated fruit generally decreased for later harvest dates (Table 5-1). On both dates, the earliest treatment increased peel rupture force by ca. 10%, but had little or no effect on τ_{st} or G presumably because of random variation in peel thickness. Rupture force was not related to peel thickness ($r^2 \leq 0.01$), but the random variation in thickness may have caused such large variation in τ_{st} and G that reported values from different treatments were not statistically different. The flavedo normally consists of a few cell layers, but albedo thickness is highly variable and may account for most of the peel's thickness (Spiegel-Roy and Goldschmidt, 1996). Therefore, variation in albedo thickness may have little effect on rupture force, while confounding interpretation of treatment effects on τ_{st} and G. These data also suggest that the flavedo is responsible for most of the peel's resistance to shear forces and that it is the tissue that GA_3-treatment affects. Shaving off layers of

Table 5-1. Shear test of 'Valencia' orange peel. Peak rupture force (F), shear strength (τ_{st}), and shear modulus (G) of peel from fruit treated with GA_3 on different dates or non-treated (control). Fruit were harvested on 23 Apr. and 13 May 1999. Peel pieces were subjected to axial loading with a 0.0254 m diameter cylindrical cutter attached to a 50 kN load cell with a cross-head speed of 0.00016 $m \bullet s^{-1}$ ($10\ mm \bullet min^{-1}$).

GA_3 application date, 1998	Harvest date, 1999					
	23 Apr.			13 May		
	F (N)	τ_{st} (kPa)	G (kPa)	F(N)	τ_{st} (kPa)	G (kPa)
25 Sept.	156.29^{z} a^{y}	588.88 a	675.73 a	147.48 a	547.53 ab	709.66 a
12 Nov.	146.58 ab	581.54 a	745.68 a	139.81 ab	580.48 a	706.62 a
8 Dec.	147.19 ab	558.62 a	717.61 a	135.40 b	500.42 b	666.86 a
Control	139.54 b	528.99 a	700.72 a	135.16 b	493.54 b	627.58 a

^{z}Values are treatment means of 11 trees/treatment. Values for each tree were the average of 5 tests/tree (1 test/fruit).
^{y}Means followed by a different letter are significantly different by Duncan's New Multiple Range Test, $P \leq 0.05$.

albedo (Coggins and Lewis, 1965) could create peel pieces of uniform thickness, but it might be more appropriate to simply measure rupture force.

When harvested late season, the peel of fruit treated at color break had 15% greater τ_{st} than the peel of fruit treated after color break or non-treated (Table 5-1). There were no other treatment effects on peel shear properties. These data are consistent with GA_3 effects on other properties related to peel deterioration such as the decrease in peel puncture resistance, and the incidence and severity of peel puffing and creasing. Each of these properties was best controlled by GA_3 applied before or at color break (Fidelibus et al., 2002b; Monselise et al., 1976; Greenberg et al., 1992). When applied at certain times GA_3 appears to slow, but not reverse, initiation and expansion of intercellular space in the flavedo and albedo (Coggins, 1969). Increased intercellular space results in less cell wall material per volume of tissue (Harker et al., 1997) and less contact area between cells (Schneider, 1969). Both factors could cause the tissue to be less resistant to shear stress (Harker et al., 1997).

Peel Tensile Test

Peel rupture force, tensile strength (σ_t), and modulus of elasticity (E_T) generally decreased as a result of delayed harvest (Table 5-2), as did shear properties. As observed in shear tests, rupture force was more affected by the treatments than σ_t, indicating that the different tissues that compose the peel are not equivalent with respect to tensile properties. This agrees with the findings of Kaufmann (1970), who showed that flavedo was more resistant to tensile force than albedo, and Garcia-Luis et al. (2001), who found that peel thickness was not correlated with incidence of fruit splitting. These findings

Table 5-2. Tension test of 'Valencia' orange peel. Rupture force (F_r), tensile strength (σ_t), and modulus of elasticity (E) of the peel of 'Valencia' orange fruit treated with GA_3 on different dates or non-treated (control). Fruit were harvested on 23 Apr. and 13 May, 1999. Peel pieces were secured with clips that anchored one end of each peel strip to the base of the testing machine and the other end to a 50 kN load cell with a cross-head speed of 0.00016 m • s^{-1} (10 mm • min^{-1}).

GA_3	Harvest date					
application	23 Apr.			13 May		
date, 1998	F_r (N)	σ_t (kPa)	E (kPa)	F_r (N)	σ_t (kPa)	E (kPa)
25 Sept.	61.65[z] a[y]	741.92 a	4002.61 ab	56.25 a	642.21 a	4194.94 a
12 Nov.	58.40 a	685.77 ab	3922.37 ab	53.84 a	625.36 a	3526.85 b
8 Dec.	56.75 ab	672.67 ab	3828.12 b	53.70 a	585.93 ab	3725.30 ab
Control	52.49 b	620.33 b	4345.53 a	46.86 b	541.39 b	3489.65 b

[z]Values are treatment means of 11 trees/treatment. Values for each tree were the average of 5 tests/tree (1 test/fruit).
[y]Means followed by a different letter are significantly different by Duncan's New Multiple Range Test, $P \leq 0.05$.

suggest that albedo thickness may have very little role in governing mechanical strength of the peel of mature oranges.

Several treatments increased tensile properties of the peel, but like shear properties, treatment effects were less for mid-season harvest than late season harvest. The peel of fruit treated before color break had about 15% greater rupture force and σ_t than the peel of non-treated fruit when harvested mid-season. The peel of fruit treated at color break also had greater rupture force during tensile testing but not greater σ_t than the peel of non-treated fruit. The peel of fruit treated after color break had a smaller E_T than non-treated fruit. On the later harvest, all treated fruit had 10-20% greater rupture force, in tension, than non-treated fruit. Moreover, the peel of fruit treated on or before color break had about 15% greater σ_t than peel of non-treated fruit, and the peel of fruit treated before color break had 17% greater E_T than non-treated fruit.

Factors that likely contributed to greater resistance to shear force (e.g., more cell wall material/volume of tissue and greater contact area between cells) might also have increased resistance to tension. However, all treatments increased rupture force in tension at mid-season harvest, but only the pre-color break treatment increased rupture force in shear, suggesting that some factors contributing to peel tensile strength are different than those related to peel shear strength. Tension tests of peel samples with different water potentials showed that tissues with the least negative water potential had the highest elastic modulus (Kaufmann, 1970). However, there are no reports of GA_3-treatment effects on the water potential of citrus peel.

Compression Test of Whole Fruit

Modulus of elasticity (E) decreased about 10% due to delayed harvest (Table 5-3). Miller (1986) also found that E decreased as the fruit aged; however, the values of E found in this study were 1/3 to 1/2 those reported by Miller (1986). These differences might be related to fruit orientation during loading. Miller (1986) and Churchill (1980) placed the fruit axis perpendicular to the plate. In this study the fruit axes were oriented parallel to the plate because 'Valencia' oranges, which have a slightly longer axial than equatorial diameter, usually fall into juice extraction cups oriented more or less horizontally (Fidelibus, pers. obs.). Because the cells and tissues of fruits are arranged relative to the fruit axis, orientation of the fruit relative to the applied force can affect its mechanical behavior (Harker et al., 1997). This could be especially true of citrus that have segments clustered around the central axis and a peel that varies in thickness from the stem to the stylar end (Schneider, 1969).

Treatments had no affect on E, a finding that is somewhat surprising considering that one of the most consistently reported effects of GA_3 on citrus is increased fruit firmness (Coggins, 1969; Davies, 1986). However, horticulturists consider firmness to be synonymous with puncture resistance (Harker, 1997). Although GA_3-treatments increased puncture resistance (Fidelibus et al., 2002b), they did not affect the stiffness or rigidity of the whole fruit. Whole fruit compression tests are often considered relatively insensitive to changes in fruit properties because fruit morphology, size, shape and turgor are highly variable (Harker et al., 1997). The complex anatomy of citrus fruit might further confound measurements of whole-fruit mechanical properties.

Table 5-3. Compression test of 'Valencia' orange fruit. Modulus of elasticity (E), degree of elasticity (D_e), mechanical hysteresis (Hyst.) and resilience (Resil.) of fruit treated with GA_3 on different dates or non-treated (control). Fruit were harvested on 23 Apr. and 13 May 1999. Fruit were supported by an aluminum plate with a 0.075 m hemispherical depression and subjected to a 0.01 m displacement by a flat plate attached to a 50 kN load cell with a cross-head speed of 0.00016 m • s^{-1} (10 mm • min^{-1}).

GA_3	Harvest date, 1999							
application	23 Apr.				13 May			
date, 1998	E (kPa)	D_e	Hyst. N • m^{-1}	Resil. N • m^{-1}	E (kPa)	D_e	Hyst. N • m^{-1}	Resil. N • m^{-1}
25 Sept.	619.74[z] a[y]	0.70 a	0.45 a	0.27 a	494.19 a	0.69 ab	0.38 a	0.21 a
12 Nov.	586.42 a	0.70 a	0.43 a	0.27 a	482.42 a	0.69 ab	0.37 a	0.21 a
8 Dec.	585.89 a	0.70 a	0.43 a	0.27 a	467.06 a	0.70 a	0.35 a	0.21 a
Control	571.88 a	0.68 a	0.42 a	0.24 a	477.91 a	0.67 b	0.36 a	0.20 a

[z]Values are treatment means of 11 trees/treatment. Values for each tree were the average of 5 tests/tree (1 test/fruit).
[y]Means followed by a different letter are significantly different within columns by Duncan's New Multiple Range Test, $P \leq 0.05$.

Degree of elasticity was the only property measured that did not appreciably decrease between harvests (Table 5-3). Treated fruit had similar D_e to control fruit except on the second harvest, when fruit treated after color break had greater D_e than non-sprayed fruit. This is in contrast with most other variables that were increased by the earliest but not the latest GA_3 application. Both hysteresis and resilience decreased to the same extent between harvests, consistent with the decrease in E (Table 5-3). Therefore, fruit became less stiff, and could not store or absorb as much strain energy as they aged.

Cutting Test of Whole Fruit

When force was applied to the fruit with the circular cutter, the fruit deformed until the cutter ruptured the peel. Therefore an apparent modulus of elasticity (E_a) was calculated from the resulting stress vs. strain charts (Fig. 5-4). In contrast to flat plate loading, loading with the circular cutter confined the force to a smaller and constant area and eliminated the problem of circle of contact. Instead of the curvilinear force and deformation relationship observed with flat-plate loading (Fig. 5-3), loading with the circular cutter resulted in a linear relationship between stress and strain (Fig. 5-4). However, values determined for E_a were about four times that calculated using a flat plate. Between harvests, E_a decreased about 20% for treated and about 17% for non-treated fruit, in contrast with E, which decreased about 10% between harvests. As observed for many properties, treatments did not affect E_a on the first harvest, but on the second harvest, fruit treated before color break had about 10% greater E_a than fruit treated on other dates.

Cutting strength of the intact peel and fruit rupture force decreased about 20 and 6 %, respectively, between harvests (Table 5-4), as observed for detached peel pieces.

Table 5-4. Cutting test of whole 'Valencia' orange fruit. Apparent modulus (E_a), peel puncture strength (PS) and rupture force (F_r) of fruit treated with GA_3 on different dates or non-treated (control). Fruit were harvested on 23 Apr. and 13 May 1999. Fruit were supported by an aluminum plate with a 0.075 m hemispherical depression and subjected to loading by a circular cutter (0.25 m diam.) attached to a 50 kN load cell with a cross-head speed of 0.0008 m • s^{-1} (50 mm • min^{-1}).

GA_3	Harvest date, 1999					
application	23 Apr.			13 May		
date, 1998	E_a (kPa)	PS (kPa)	F_r (N)	E_a (kPa)	PS (kPa)	F_r (N)
25 Sept.	2618.7[z] a[y]	621.9 a	167.3 a	2063.3 a	570.4 a	157.2 a
12 Nov.	2419.4 a	616.6 a	160.8 a	1909.6 b	536.8 a	144.5 b
8 Dec.	2295.8 a	611.1 a	158.2 a	1872.7 b	537.1 a	143.6 b
Control	2313.8 a	609.8 a	153.9a	1967.0 ab	551.1 a	144.4 b

[z]Values are treatment means of 11 trees/treatment. Values for each tree were the average of 5 tests/tree (1 test/fruit).
[y]Means followed by a different letter are significantly different by Duncan's New Multiple Range Test, $P \leq 0.05$.

Although puncture strength and rupture force were only slightly higher for intact vs. detached peel pieces, tests on detached pieces were more sensitive to treatment effects than were intact pieces. As observed for most variables, there were no treatment effects mid-season. Late season, fruit treated before color break had greater rupture force than all other treatments.

Conclusions. In general, GA_3-treatment before color break considerably increased shear and tensile properties of the peel and slightly increased fruit puncture force and apparent modulus of elasticity (E_a) but generally did not affect the modulus of elasticity (E) or the degree of elasticity (D_e). Mechanical properties of the peel correlated poorly with peel thickness, presumably because the albedo contributed the most to peel thickness, while the flavedo seems to be responsible for most of the peel's strength and to be most affected by GA_3 application. Gibberellic acid was most effective if applied before color break. This is probably because the treatment delays, but does not reverse, physical changes that accompany peel maturation. Because the earliest treatment application was the most effective, mechanical properties of the peel might be further improved by GA_3 applications that are earlier than those tested. Treatment effects were less evident at mid-season than at late-season, when peel senescence was more pronounced.

CHAPTER 6
GIBBERELLIC ACID APPLICATION INCREASES JUICE YIELD BY REDUCING PEEL VOLUME

Introduction

Practices that increase juice yield are of considerable interest in Florida where > 95% of the crop is used for juice (Anonymous, 2001). Recently, Fidelibus et al. (2002b) showed that GA_3 applied to sweet oranges at about color break increased juice yield about 5%. How GA_3 application increased juice yield is not known.

Processors observed that GA_3-treated fruit were more resistant than non-treated fruit to bursting during juice extraction when harvested late season (J. Keithley, pers. comm.); this response might improve juice extraction efficiency by helping to retain pulp in the juice stream (Fidelibus et al., 2002b). In support of this hypothesis, Davies et al. (1997) found that peel puncture resistance, an indicator of the mechanical integrity of the peel, was increased by GA_3 treatment and highly correlated with juice yield. However, batches of fruit with different peel puncture resistance had similar juice yield (Appendix A). Moreover, GA_3 treatment increased peel shear and tensile strength by 10-20% (Fidelibus et al, 2002a), but these and other mechanical properties of the peel and whole fruit were not correlated with juice yield. In addition, GA_3 increased juice yield most relatively early in the season when non-treated fruit retained reasonably good peel integrity (Fidelibus et al., 2002b).

An alternative hypothesis is that GA_3 treatment increased the amount of pulp in the fruit or the pulp/peel ratio. Most studies have reported that GA_3 treatment does not

affect the amount of pulp in the fruit (Coggins, 1968; Davies, 1986), but the effect of GA_3 treatment on peel growth is inconsistent. Sometimes, a single application of GA_3 at about color break resulted in a more compact peel (Coggins, 1968; Pozo et al., 2000). In contrast, continuously high levels of GA_3 may result in fruit with unusually thick peels (Goldschmidt, 1983; Erner et al., 1976). In any case, peel thickness is not necessarily correlated with juice yield (Fidelibus et al., 2002a).

A third hypothesis is that application of GA_3 might alter water exchange between the fruit and tree, as proposed by Garcia-Luis et al. (1985), or reduce evaporation from the fruit surface (Fucik, 1981). If either hypothesis is correct, then the peel of GA_3-treated fruit would have a higher peel water content (PWC) than non-treated fruit because water loss is primarily from the peel rather than the pulp (Rokach, 1953). Therefore, knowledge of PWC might be of potential use to growers for scheduling harvests. However, measurement of PWC is relatively time consuming. Water status of the peel might be inferred by gap width, a measure of the distance between the cut edges of the peel in the slice gap that develops after fruit are cut mid way through the equator (Kaufmann, 1970).

The purpose of this experiment was to characterize the effect of GA_3 treatment on peel growth and juice yield. A secondary objective was to test whether gap width might be a useful indicator of peel water content, juice yield, or GA_3 efficacy.

Materials and Methods

Fruit used in this study were from 'Hamlin' sweet oranges [*Citrus sinensis* (L.) Osb.] budded on Carrizo citrange [*Citrus sinensis* (L.) Osb. x *Poncirus trifoliata* (L.) Raf.] rootstock. Trees were about 20-years-old and planted in a commercial grove near Groveland, Fla.

Ten trees were selected based on uniform tree size, vigor, crop-load and position in the grove. Two west-facing branches, bearing a similar amount of fruit, were selected on each tree. A coin toss was used to select one branch per tree for GA_3 treatment and the other was marked as a non-treated control. On 2 Oct. 2001, fruit and foliage were treated with a solution of GA_3 (45 g a.i. ha-1; ProGibb®, Valent, Libertyville, Ill.) and an organo-silicone surfactant (0.05%, v/v; Silwet®, Helena Chemical Corp., Memphis, Tenn.) applied until runoff with a hand-held sprayer (Solo, Newport News, VA). Non-treated branches were protected by plastic sheeting during treatment application.

Fruit were harvested ca. every 7-14 d until 19 Dec. 2001. Three fruit per branch were collected at each harvest. Harvested fruit were brushed clean with a soft cloth and a Minolta chroma meter (Minolta, Ramsey, N.J.) was used to measure peel color (hue angle) at three equidistant points around each fruit equator (Fidelibus et al., 2002b). Fruit equatorial diameter was measured with calipers. Fresh weight of each three fruit sample was recorded, and each fruit was then sliced halfway across the fruit equator and the maximum gap width between the edges of the cut peels was measured with calipers (Kaufmann, 1970). Peel thickness was measured with calipers. Peel volume was estimated as the difference between fruit and pulp volume, where each volume was calculated as a sphere (vol. = $4/3 \bullet \pi \bullet r^3$). The fruit radius, r, was half its equatorial diameter, while the radius of the pulp was considered to be equal to the fruit radius minus the peel thickness. Each three fruit sample was juiced with a reamer and fresh weight of the peel and juice was recorded. The peels were re-weighed after drying in an oven (60 °C) for 3 d, and peel water content was calculated as the ratio of dry to wet weight.

Data were subjected to analysis of variance by date using PROC GLM (SAS, SAS Inst., Cary, N.C.). Regression analysis (PROC REG, SAS) was used to test relationships between variables and time.

Results and Discussion

Application of GA_3 had a rapid effect on peel color and changed the rate at which green peel color decreased for the duration of the experiment. The peel of GA_3-treated fruits became less green (peel hue angle decreased) at about half the rate of non-treated fruit (Fig. 6-1) such that treatment effects on peel color were obvious within 21 d after treatment (DAT). Peel color was the variable affected earliest by GA_3 application; effects of GA_3 on other measured variables were not observed until at least 35 DAT.

Juice yield of treated and non-treated fruit increased over time (Fig. 6-2). On most dates, treated and non-treated fruit had similar juice yield, but on 58 DAT, treated fruit had slightly (about 3%, standardized to control), but significantly, greater juice yield than non-treated fruit. On 77 DAT, the difference between the mean juice yield of GA_3-treated and non-treated fruit was about the same as on 58 DAT, but the variation was greater so GA_3 did not significantly increase juice yield on 77 DAT. This agrees with earlier findings (Fidelibus et al., 2002b) that 'Hamlin' oranges treated with GA_3 in late September or October had 3-5% greater juice yield than non-treated fruit when harvested in December or January, although the duration of the yield increase was shorter. The lesser effect of GA_3 application on juice yield reported here might be partly explained by

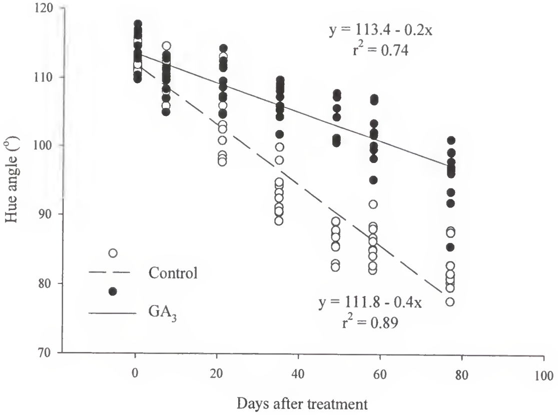

Fig. 6-1. Peel hue angle as a function of harvest date for fruit treated with GA_3 or non-treated, Groveland, Fla., 2001.

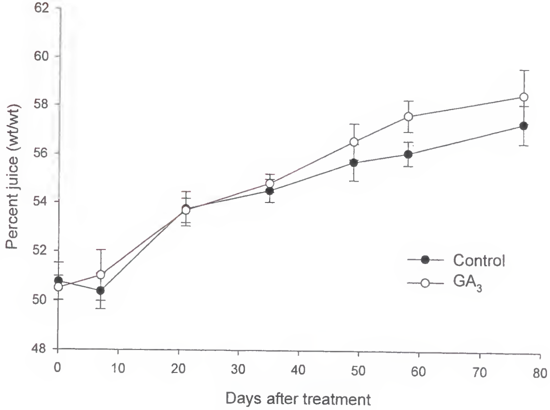

Fig. 6-2. Effect of 2 Oct. 2001 GA_3 application on mean percent juice of 'Hamlin' oranges, Groveland, Fla. Means ± SE, n=10 for each harvest date.

the fact that fruit were juiced with a reamer rather than a commercial machine. The extraction efficiency of these methods is different and varies according to the condition and size of the fruit (Sinclair, 1972).

Percent peel volume was somewhat variable; however, it generally remained about the same for non-treated fruit and decreased for GA_3 treated fruit (Fig. 6-3). In fact, between 35 and 77 DAT, GA_3-treated fruit had significantly less percent peel volume than non-treated fruit. Apparently, peel volume of non-treated fruit remained about the same because peel thickness increased slightly (about 5-10%; data not shown) in non-treated fruit over the course of the experiment. In contrast, peel volume decreased in GA_3-treated fruit because peel thickness remained the same or slightly decreased (data not shown) while pulp growth increased, as evidenced by increasing juice yield (Fig. 6-2). Similarly, Garcia-Luis et al. (1985) and Pozo et al. (2000) reported that application of GA_3 to mandarins (*Citrus reticulata* Blanco) at color break suppressed subsequent peel thickening.

Increased juice yield of GA_3-treated fruit was likely the result of these fruit having lower percent peel volume than non-treated fruit. For example, on day 58, when GA_3-treated fruit had significantly greater juice yield than non-treated fruit, there was an inverse, linear relationship between percent juice, by weight, and percent peel volume (Fig. 6-4). These data agree with those of Garcia-Luis et al. (1985) and Pozo et al. (2000) who found that GA_3 application increased juice yield and decreased peel thickness of mandarins. Because the peel has a lower specific gravity than the pulp, juice yield on a weight basis may be less accurate than yield reported on a volume basis (Sinclair, 1972) which could partly explain why GA_3-treated fruit often had higher mean

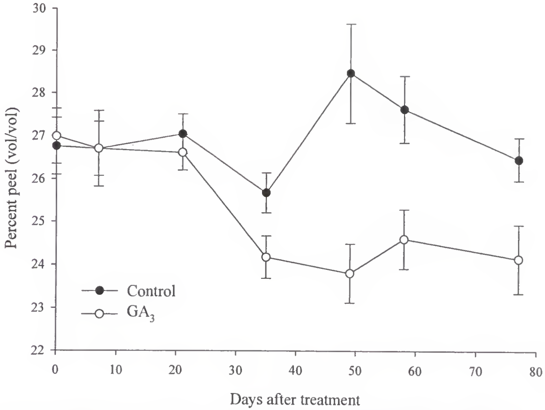

Fig. 6-3. Effect of 2 Oct. 2001 GA_3 application on mean percent peel volume of 'Hamlin' oranges, Groveland, Fla. Means $\pm$ SE, n=10 for each harvest date.

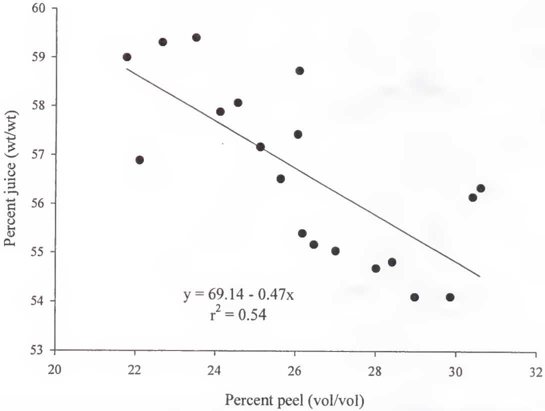

Fig. 6-4. Percent juice and percent peel volume of fruit treated with GA_3 and non-treated 59 DAT, Groveland, Fla., 2001.

juice yield by weight than non-treated fruit even though such differences were not always statistically significant (Fig. 6-2.; Fidelibus et al., 2002b; Davies et al., 1997).

Gap width between GA_3-treated and non-treated fruit varied widely throughout the experiment (Fig. 6-5), and there was no obvious relationship between gap width and harvest date. However, the gap widths of non-treated fruits were significantly smaller than those of GA_3-treated fruits between 35 and 77 DAT. The only variable measured that was more sensitive to GA_3 application than gap width was peel color. Therefore, gap widths, which are easy to measure and do not require special equipment, might be useful alternatives to other measurements of GA_3 effects on peel mechanical integrity, such as peel puncture resistance.

Gap width was not related to juice yield, and GA_3 application did not affect PWC on most dates (data not shown). However, for non-treated fruit, there was a moderate, positive linear relationship between gap width and PWC ($r^2 = 0.41$). In contrast, the relationship between gap width and PWC was much less distinct for fruit treated with GA_3 ($r^2 = 0.24$). Therefore, gap width responds to PWC and some other variable or variables that are affected by GA_3 application.

Slicing an orange releases tension stress borne by the peel, so gap width should be proportional to the stress being released (Kaufmann, 1970). Therefore, the peels of GA_3-treated fruits were probably bearing more stress than the peels of non-treated fruit. Fidelibus et al. (2002a) showed that GA_3 treatment increased the peels' modulus of elasticity in tension, so more of the stress imposed on the peel by pulp growth would be stored in GA_3-treated fruit. In non-treated fruits, some of the stress on the peel is the

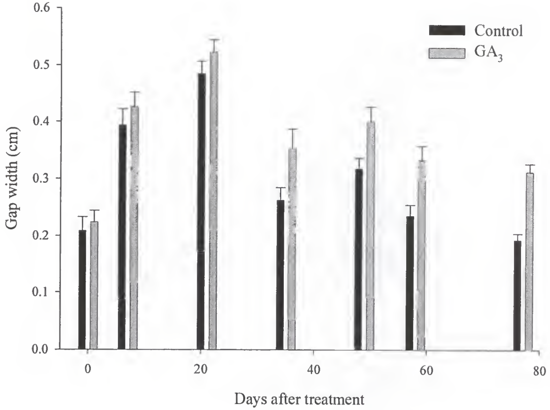

Fig 6-5. Effect of 2 Oct. 2001 GA_3 application on mean gap width between the edges of the peel of 'Hamlin' oranges sliced half way through the fruit equator, Groveland, Fla. Means $\pm$ SE, n=10 for each harvest date.

result of turgor pressure (Kaufmann, 1970); hence the moderate relationship between PWC and gap width.

Conclusions. Fruit treated with GA_3 at about color break lost green peel color at half the rate of non-treated fruit. Application of GA_3 enhanced juice yield, but the effect was slight and of short duration. Gibberellic acid apparently increased juice yield by reducing peel volume. Juice yield was not related to PWC. Gap width was a reasonable indicator of PWC for non-treated fruit but not for GA_3-treated fruit, probably because the treatment increased peel tensile strength independent of PWC. Although gap width was not related to juice yield, it is easy to measure, responsive to GA_3 treatment, and thus might be a good indicator of GA_3 efficacy.

CHAPTER 7
GIBBERELLIC ACID APPLICATION AFFECTS SOLUBLE SUGAR LEVELS AND COLOR OF ORANGE PEELS

Introduction

In the fall or winter, the colored portion of the peel (flavedo) of sweet orange fruit changes color from green to yellow. This process, known as color break, involves conversion of chloroplasts to chromoplasts and is stimulated by low temperatures (Erickson, 1968). The physiology of color break is not fully understood but it appears to be modulated by peel sugars (Goldschmidt and Koch, 1996). For example, low temperature (5° C) stimulated invertase activity and increased levels of reducing sugars in grapefruit flavedo (Purvis and Rice, 1983), and increased levels of soluble sugars corresponded with color break of 'Valencia' orange peel (Huff, 1984). Moreover, peel pieces in culture degreened when sucrose levels of the media were increased and regreened when sucrose was not provided (Huff, 1984). Likewise, regreening of 'Valencia' orange, which occurs in the spring (Thomson et al., 1967), is preceded by a decrease in peel soluble sugars (Huff, 1984). Recently, it was demonstrated that peel sugar levels could be increased and color break advanced by injection of sucrose into phloem subtending the fruit (Iglesias et al., 2001). All of these observations are consistent with the theory that high levels of soluble sugars down-regulate genes encoding photosynthetic products (Koch, 1996).

Gibberellic acid (GA_3) is applied to citrus in the late summer or fall, primarily to improve peel integrity and extend the harvest season (Coggins, 1969). However, such

applications also retard color break and green peel color may further delay harvest. Because peel color is an important factor determining fresh fruit quality (Davies, 1986), it is desirable to understand the physiology of GA_3 effects on fruit color.

The mechanism by which GA_3 delays color break is unknown, but it seems likely that it might affect soluble sugar levels of the peel or the ability of peel tissues to sense sugars. There are precedents for growth regulators to have both effects. For example, GA_3 is widely used in brewing because it stimulates starch metabolism of seeds (Martin, 1983), and auxin inhibited sucrose modulation of sugar sensitive genes of soybean (DeWald et al., 1994). In a sucrose feeding study, Iglesias et al. (2001) found that GA_3 treatment did not affect peel color in the absence of an elicitor such as sucrose. The authors suggested that GA_3 treatment might act by repressing an ethylene response that is stimulated by high sucrose levels. However, they did not show whether GA_3 treatment affected flavedo sugar levels, or whether the levels of other carbohydrates such as glucose or fructose were affected by sucrose feeding.

Recently, it was shown that GA_3 application timing affected peel color and juice Brix of sweet oranges such that the application times that best maintained green peel color also caused a reduction in juice Brix (Fidelibus et al., 2002b; Appendix B). It is unknown how GA_3 treatment could affect juice Brix, but as in the case of peel color, sugar levels might be involved. In grapefruit, sugars followed an ascending gradient from peel to pulp until about color break and a descending gradient after color break (Koch and Avigne, 1990). Therefore, high sugar levels in the peel might enhance sugar accumulation in the pulp. If GA_3 treatment reduced peel sugars, peel color and juice Brix could both be affected. The purpose of this study was to test whether GA_3 application

affected peel sugar levels and whether levels of peel sugars were related to effects on peel color and juice Brix.

Materials and Methods

Fruit used in this study were from mature 'Hamlin' sweet orange [*Citrus sinensis* (L.) Osb.] trees grown in Gainesville (2000) or Groveland, Fla (2001). The trees in Gainesville were 17-years-old and budded on sour orange (*Citrus aurantium* L.) rootstock. Trees in Groveland were about 20-years-old, and budded on Carrizo citrange [*Citrus sinensis* (L.) Osb. x *Poncirus trifoliata* (L.) Raf.] rootstock planted in a commercial grove.

On 6 Oct 2000, and 2 Oct. 2001, four trees were selected based on uniformity of size, vigor, crop-load, and position in the grove. Two west-facing branches bearing a similar amount of fruit were selected on each tree. A coin toss was used to select one branch per tree for GA_3 treatment and the other branch was marked as a non-treated control. The treatment consisted of a solution of GA_3 (45 g a.i. ha^{-1}; ProGibb, Valent, Libertyville, Ill.) and an organo-silicone surfactant (0.05%, v/v; Silwet, Helena Chemical Corp., Memphis, Tenn.) applied to the fruit and foliage of the treated branch with a hand-held sprayer (Solo, Newport News, VA) until runoff. Non-treated branches were protected by plastic sheeting during treatment application.

Respiration measurements. Five fruit per branch were harvested from treated and non-treated branches when non-treated fruit were mostly green (G), pre-color break (PCB), at color break (CB) and when fully yellow (Y; Table 1). Harvest dates were as follows; G, 2 d after treatment (DAT), 2000 and 2001; pre-color break, 7 DAT, 2001; color break 12 DAT, 2000, 21 DAT 2001, and fully yellow 54 DAT, 2000, 58 DAT, 2001. Immediately after harvest, fruit were surface sterilized by gently washing them in

a 10% solution of sodium hypochlorite. The fruit were air dried, weighed, and peel color was measured with a Minolta chroma-meter (Fidelibus et al., 2002). Each five-fruit sample was then placed in 5 L glass jars and stored in the dark at 20 °C and 80% RH for 24 h. The jars were then sealed for 1 h, and respiration rate was calculated from the [CO_2] in the headspace as measured by a gas chromatograph (Gow-Mac Instrument Co., Bridgewater, N.J.) after 1 h.

Soluble sugar analysis. After fruit respiration measurements, a sample of flavedo, and albedo was collected from each set of fruit. In 2000, three peel discs (1.27 cm diam.) were removed from the equator of three of the four five-fruit samples (trees) per treatment. Three of the four trees were selected for carbohydrate analysis at random on the first harvest and then sugar data were collected from fruit of the same three trees at each subsequent harvest. In 2001, 10 peel discs per fruit were excised from the four five-fruit samples at the first three harvests. However, sugar data were collected from three five-fruit samples per treatment on the fourth harvest to accommodate juice samples. Flavedo was separated from albedo with a scalpel and the tissues were immediately frozen in liquid nitrogen and stored at –80 °C until further analysis. In 2000, a 2.54 cm length of pulp segment underlying each peel sample was removed with a scalpel and frozen in liquid nitrogen. In 2001, juice was extracted from the fruit with a reamer and stored at –20 °C until further analysis.

Each sample of frozen tissue was ground with a mortar and pestle to a fine powder under liquid nitrogen and prepared for analysis by HPLC following the methods of Baldwin et al. (1991). The powdered tissue of each sample was homogenized and a 5 g subsample was added to 10 ml of 80% ethanol, boiled for 15 min (with a loose-fitting

cover), cooled, and vacuum-filtered through Whatman #4 filter paper. The extract was brought up to 10 ml with 80% ethanol and passed through a C-18 Sep Pak cartridge (Waters/Millipore, Milford, Mass.) and a 0.45 m Millipore filter. The filtered extract was injected into a Perkin-Elmer series 410 HPLC system (Perkin-Elmer, Norwalk, Conn.). Levels of fructose, glucose and sucrose were determined using a Waters Sugar Pak column at 90° C with a mobile phase of 100 mM ethlenediamine-tetraacetic acid disodium-calcium salt (CaEDTA) and a flow rate of 2 ml min^{-1}.

Juice was thawed, mixed and allowed to come to room temperature (25° C). Brix of the juice was measured with a refractometer (Bellingham and Stanley Ltd., Kent, United Kingdom). Total acid was determined by titration with 0.3125 N NaOH and soluble solids content was determined by adjusting the Brix for total acids. When treatment affects on juice Brix were observed (58 DAT 2001) about 5 ml of juice of three treated and non-treated samples was retained to determine sugar composition.

Data were subjected to analysis of variance using PROC GLM (SAS Institute Inc., Cary, N.C.) and treatment means and SE were calculated using PROC MEANS (SAS). All data were subjected to regression analysis using PROC REG (SAS) to examine the relationships between variables.

Results and Discussion

Peel color. Peel color break commenced more quickly in 2000 (12 DAT) than 2001 (21 DAT), probably because temperatures were unseasonably cool during 2000 (data not shown). In 2000 and 2001, when non-treated fruit were at color break, or fully yellow, treated fruit were more green than non-treated fruit (Table 7-1). Therefore, GA_3 had a rapid effect on peel color.

Fruit respiration. In general, fruit respiration was low, 5-12 mg $CO_2 \bullet kg^{-1} \bullet h^{-1}$, and there were no treatment effects (Appendix C). These data are consistent with those of Lewis et al. (1967) who found that the peel of GA_3 treated and non-treated fruit had similar respiration rates in the fall and winter. Fruit respiration was not strongly correlated with any other variables measured.

Flavedo soluble sugar levels. Flavedo fructose levels in non-treated fruit were about the same (7-10 mg $\bullet g^{-1}$) for fruit that were green or at color break, but fruit with yellow peels had 40-50% greater flavedo fructose levels than other fruit (Table 7-2). In contrast, flavedo fructose levels in treated fruit at color break, 2000, and pre-color break and color break 2001, were less than those of green fruit such that treated fruit had slightly to substantially less fructose than non-treated fruit on these dates. Fructose levels in treated fruit were 40-60% greater for yellow fruit than fruit at color break, as observed in non-treated fruit, but in both years, flavedo fructose levels of treated fruit remained about 25% lower than non-treated fruit at the last harvest.

Flavedo glucose levels were generally greater than those of fructose (Table 7-3). As observed for fructose, flavedo glucose levels in non-treated fruit were slightly less for fruit at color break than for green fruit, while yellow fruit had about 30% more glucose than fruit at color break. A similar trend was observed in grapefruit, where reducing sugars decreased slightly from October to November before increasing markedly in December and January (Purvis and Rice, 1983). In general, treated fruit generally had lower flavedo glucose levels than non-treated fruit when harvested pre-color break, color

Table 7-1. Peel hue angle of 'Hamlin' orange fruit treated with GA_3 on 6 Oct. 2000 or 2 Oct. 2001, or non-treated, and harvested when non-treated fruit were green (G), pre-color break (PCB), at color break (CB), or fully yellow (Y), 2000, Gainesville, Fla. and 2001, Groveland, Fla.

	Year						
	2000			2001			
	Fruit developmental stage[z]						
	G[y]	CB	Y	G	PCB	CB	Y
Treatment	Peel hue angle (°)						
Non-treated	114.2[x]	103.9	84.6	114.3	114.1	110.0	86.8
GA_3	112.4	108.6	92.0	114.5	113.2	113.8	103.8
Significance[w]	0.98	0.08	0.05	0.89	0.23	0.05	0.01

[z]Fruit developmental stage was a qualitative stage based on the appearance of non-treated fruit at each harvest.

[y]Harvest dates were as follows; G = 2 d after treatment (DAT), 2000 and 2001; PCB = 7 DAT, 2001; CB = 12, 2000, and 21, 2001, and Y = 54 DAT, 2000, and 58 DAT, 2001.

[x]Values are treatment means based on a sample of five fruit from each of three (2000 and Y, 2001) or four (2001) trees per treatment. Three measurements were made equidistant around the fruit equator.

[w]$P \leq F$, as determined by a t-test within columns.

Table 7-2. Flavedo fructose levels of 'Hamlin' orange fruit treated with GA_3 on 6 Oct. 2000 or 2 Oct. 2001, or non-treated, and harvested when non-treated fruit were green (G), pre-color break (PCB), at color break (CB), or fully yellow (Y), 2000, Gainesville, Fla. and 2001, Groveland, Fla.

	Year						
	2000			2001			
	Peel developmental stage[z]						
Treatment	G[y]	CB	Y	G	PCB	CB	Y
	Fructose (mg • g^{-1} flavedo FWT)						
Non-treated	10.4[x]	10.5	17.2	7.3	8.9	6.5	14.0
GA_3	8.2	7.8	13.0	7.6	5.6	3.5	10.1
Significance[w]	0.34	0.16	0.23	0.92	0.004	0.03	0.23

[z]Peel developmental stage was a qualitative stage based on the appearance of non-treated fruit at each harvest.

[y]Harvest dates were as follows; G = 2 d after treatment (DAT), 2000 and 2001; PCB = 7 DAT, 2001; CB = 12, 2000, and 21, 2001, and Y = 54 DAT, 2000, and 58 DAT, 2001.

[x]Values are treatment means based on a sample of five fruit from each of three (2000 and Y, 2001) or four (2001) trees per treatment.

[w]$P \leq F$, as determined by a t-test within columns.

Table 7-3. Flavedo glucose levels of 'Hamlin' orange fruit treated with GA_3 on 6 Oct. 2000 or 2 Oct. 2001, or non-treated, and harvested when non-treated fruit were green (G), pre-color break (PCB), at color break (CB), or fully yellow (Y), 2000, Gainesville, Fla. and 2001, Groveland, Fla.

	Year						
	2000			2001			
	Peel developmental stage[z]						
Treatment	G[y]	CB	Y	G	PCB	CB	Y
	Glucose (mg • g^{-1} flavedo FWT)						
Non-treated	13.4[x]	12.5	17.1	13.4	13.6	12.3	17.6
GA_3	11.3	10.5	15.4	12.1	12.8	11.6	13.3
Significance[w]	0.23	0.41	0.57	0.50	0.04	0.02	0.14

[z]Peel developmental stage was a qualitative stage based on the appearance of non-treated fruit at each harvest.

[y]Harvest dates were as follows; G = 2 d after treatment (DAT), 2000 and 2001; PCB = 7 DAT, 2001; CB = 12, 2000, and 21, 2001, and Y = 54 DAT, 2000, and 58 DAT, 2001.

[x]Values are treatment means based on a sample of five fruit from each of three (2000 and Y, 2001) or four (2001) trees per treatment.

[w]$P \leq F$, as determined by a t-test within columns.

break, and yellow. However, in 2000, a small sample size and variability in glucose levels resulted in relatively large variation. Nevertheless, the treatment means in 2000 are very similar to those in 2001, where a larger sample size helped reduce variability.

Similarly, Monselise and Goren (1965) found that two applications of GA_3 in the fall resulted in less reducing sugars in the flavedo of 'Shamouti' orange in the winter. It is unknown why GA_3 treatment reduced flavedo fructose and glucose levels, but Lewis et al. (1967) reported that GA_3 treated citrus peel utilized more glucose than non-treated peel. Data presented here do not support the theory that lower flavedo fructose and glucose levels in GA_3-treated fruit were caused by increased respiration because treated and non-treated fruit generally had similar respiration rates and respiration was poorly correlated with peel sugar levels.

Flavedo sucrose levels were variable (7.4-20.7 mg • g^{-1}), but generally increased with peel maturity (Table 7-4) as observed by others (Komatsu et al., 2002; Iglesias et al., 2001). Treatment effects were few and inconsistent. In 2000, flavedo sucrose levels of non-treated and treated fruit were similar for green and color break fruit, but yellow, non-treated, fruit had higher sucrose levels than treated fruit. In contrast, fruit treated in 2001 had lower flavedo sucrose levels than non-treated fruit, pre-color break, but higher sucrose levels when yellow. Flavedo fructose, glucose and sucrose levels measured in non-treated fruit were very similar to those reported by Komatsu et al. (2002) for flavedo of Satsuma mandarin (*Citrus unshiu* Marc.) harvested at similar stages of maturation. Total soluble sugars (fructose + glucose + sucrose) generally increased with increasing peel maturity (data not shown).

Table 7-4. Flavedo sucrose levels of 'Hamlin' orange fruit treated with GA_3 on 6 Oct. 2000 or 2 Oct. 2001, or non-treated, and harvested when non-treated fruit were green (G), pre-color break (PCB), at color break (CB), or fully yellow (Y), 2000, Gainesville, Fla. and 2001, Groveland, Fla.

	Year						
	2000			2001			
	Fruit developmental stage[z]						
	G[y]	CB	Y	G	PCB	CB	Y
Treatment	Sucrose (mg • g^{-1} flavedo FWT)						
Non-treated	8.3[x]	7.4	12.5	12.8	20.7	16.4	15.0
GA_3	8.4	8.5	11.0	13.2	16.7	14.4	17.1
Significance[w]	0.90	0.69	0.13	0.91	0.18	0.64	0.10

[z]Fruit developmental stage was a qualitative stage based on the appearance of non-treated fruit at each harvest.
[y]Harvest dates were as follows; G = 2 d after treatment (DAT), 2000 and 2001; PCB = 7 DAT, 2001; CB = 12, 2000, and 21, 2001, and Y = 54 DAT, 2000, and 58 DAT, 2001.
[x]Values are treatment means based on a sample of five fruit from each of three (2000 and Y, 2001) or four (2001) trees per treatment.
[w]$P \leq F$, as determined by a t-test within columns.

Flavedo soluble sugar levels and color. Flavedo fructose and glucose levels were often greater in non-treated than treated fruit before, or on, any day where treatments affected peel color as would be expected if sugar levels affected peel color (Huff, 1984). Between dates where treatment affected peel color in 2000 and 2001, flavedo total soluble sugar levels and green peel color had an inverse, linear relationship (Fig. 7-1), as observed by others (Huff, 1984; Iglesias et al., 2001). The relationship between peel color and soluble sugar levels appeared to be less variable at high peel hue angles (more green peel color) and low soluble sugar levels as might be expected if genes for photosynthesis were upregulated by low levels of peel soluble sugars (Koch, 1996). This agrees with observations that gibberellin activity of 'Valencia' orange flavedo increased (Rasmussen, 1973) and soluble sugar levels decreased (Huff, 1984) prior to regreening.

Of the individual soluble sugars measured, fructose (Fig. 7-2) and glucose (Fig. 7-3), but not sucrose (Fig. 7-4), had a consistent inverse relationship with peel color. An inverse relationship between sucrose and green peel color was observed in 2000, but not in 2001 (Fig. 7-4). These findings agree with the observation that sugar sensitive genes may be more responsive to hexose sugars, such as fructose and glucose, than to sucrose (Koch, 1996).

Therefore, it appears that GA_3 treatment might modulate peel color by reducing or delaying accumulation of fructose, glucose, or both, in the flavedo. This hypothesis is an alternative to that of Iglesias et al. (2001) who suggested that GA_3 treatment might delay color break by negating the stimulatory effect of sucrose on peel color break.

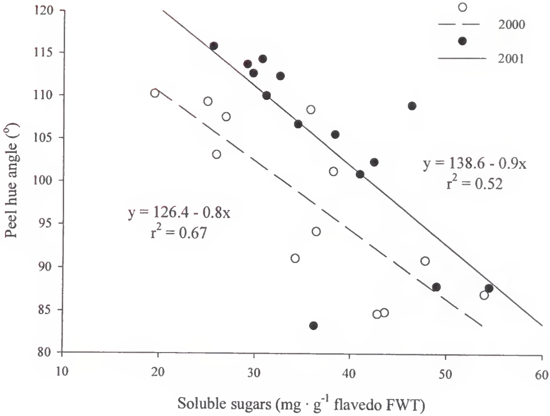

Fig. 7-1. Peel hue angle and total soluble sugars (fructose + glucose + sucrose) of 'Hamlin' orange flavedo treated with GA_3 and non-treated and harvested when non-treated fruit were at color break and fully yellow, 2000 and 2001. Color break occurred 12 and 21 d after treatment (DAT), and fruit were fully yellow 54 and 58 DAT, 2000, and 2001, respectively.

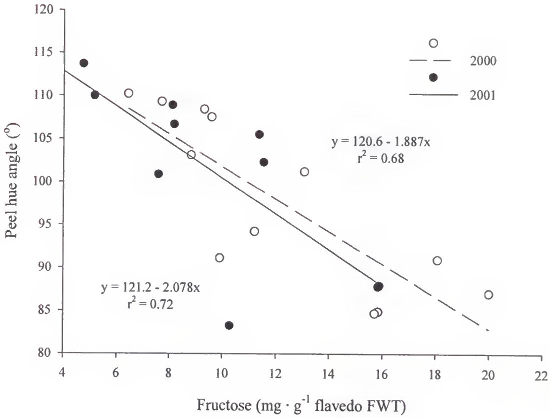

Fig. 7-2. Peel hue angle and fructose concentration of 'Hamlin' orange flavedo treated with GA_3 and non-treated and harvested when non-treated fruit were at color break and fully yellow, 2000 and 2001. Color break occurred 12 and 21 d after treatment (DAT), and fruit were fully yellow 54 and 58 DAT, 2000 and 2001, respectively.

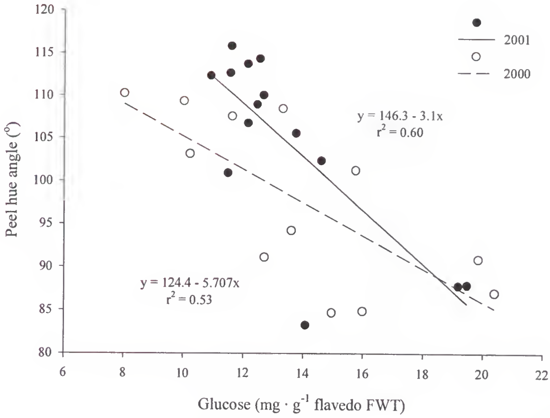

Fig. 7-3. Peel hue angle and glucose concentration of 'Hamlin' orange flavedo treated with GA_3 and non-treated and harvested when non-treated fruit were at color break and fully yellow, 2000 and 2001. Color break occurred 12 and 21 d after treatment (DAT), and fruit were fully yellow 54 and 58 DAT, 2000 and 2001, respectively.

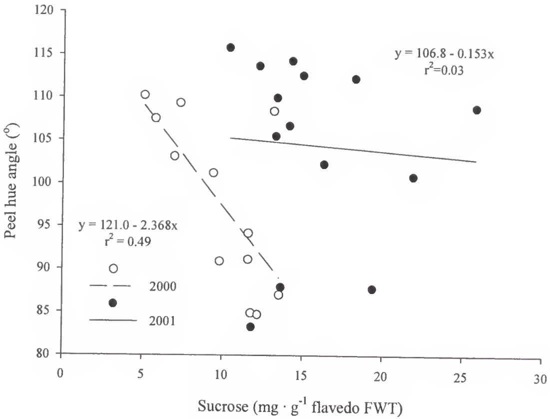

Fig. 7-4. Peel hue angle and sucrose concentration of 'Hamlin' orange flavedo treated with GA_3 and non-treated and harvested when non-treated fruit were at color break and fully yellow, 2000 and 2001. Color break occurred 12 and 21 d after treatment (DAT), and fruit were fully yellow 54 and 58 DAT, 2000 and 2001, respectively.

Albedo soluble sugars. As observed in the flavedo, fructose levels of the albedo were much greater in yellow fruit than fruit harvested at color break or earlier (Table 7-5). In 2000, GA_3 did not affect albedo fructose levels, but the albedo of treated fruit had about 40% less fructose at, or after, color break than the albedo of non-treated fruit in 2001. In contrast, albedo glucose levels of treated and non-treated fruit were between 15 and 24 mg • g^{-1} fresh weight at each harvest and there were no treatment effects (data not shown).

Sucrose levels of the albedo, in contrast with the flavedo, were generally greatest for green fruit and were less for fruit at color break or for yellow fruit (Table 7-6). However, as observed in the flavedo, GA_3 treatment in 2000 resulted in greater albedo sucrose levels than non-treated fruit at and after color break. Albedo sucrose levels of fruit treated in 2001 did not decline as the fruit degreened, so treated fruit had greater albedo sucrose levels on the last harvest, as observed in 2000.

Juice soluble solids content (SSC). Treated and non-treated fruit had generally similar total sugars in the pulp at each harvest date of 2000 (data not shown), but in 2001, when non-treated fruit were yellow, they had slightly greater SSC than treated fruit (11.3 and 10.5 %, respectively). The timing and magnitude of the GA_3-induced juice SSC reduction observed in 2001 is consistent with previous studies (Monselise and Goren, 1965; Fidelibus et al., 2002b). The composition of the juice from treated and non-treated fruit was about the same each year (50% sucrose, 30% fructose, 20% glucose). It is unknown why GA_3 treatment did not have a consistent effect on juice SSC. Ferguson et al. (1986) observed that exogenously applied GA_3 was not translocated to the peel or pulp which suggests that GA_3 effects on the pulp would have to occur indirectly via the peel.

Table 7-5. Albedo fructose levels of 'Hamlin' orange fruit treated with GA_3 on 6 Oct. 2000 or 2 Oct. 2001, or non-treated, and harvested when non-treated fruit were green (G), pre-color break (PCB), at color break (CB), or fully yellow (Y), 2000, Gainesville, Fla. and 2001, Groveland, Fla.

	Year						
	2000			2001			
	Fruit developmental stage[z]						
	G[y]	CB	Y	G	PCB	CB	Y
Treatment	Fructose (mg • g^{-1} albedo FWT)						
Non-treated	12.9[x]	11.4	15.5	8.0	6.8	7.3	20.7
GA_3	10.3	11.0	15.5	7.4	6.1	3.9	12.0
Significance[w]	0.36	0.88	0.98	0.83	0.49	0.001	0.02

[z]Fruit developmental stage was a qualitative stage based on the appearance of non-treated fruit at each harvest.
[y]Harvest dates were as follows; G = 2 d after treatment (DAT), 2000 and 2001; PCB = 7 DAT, 2001; CB = 12, 2000, and 21, 2001, and Y = 54 DAT, 2000, and 58 DAT, 2001.
[x]Values are treatment means based on a sample of five fruit from each of three (2000 and Y, 2001) or four (2001) trees per treatment.
[w]$P \leq F$, as determined by a t-test within columns.

Table 7-6. Albedo sucrose levels of 'Hamlin' orange fruit treated with GA_3 on 6 Oct. 2000 or 2 Oct. 2001, or non-treated, and harvested when non-treated fruit were green (G), pre-color break (PCB), at color break (CB), or fully yellow (Y), 2000, Gainesville, Fla. and 2001, Groveland, Fla.

	Year						
	2000			2001			
	Fruit developmental stage[z]						
	G[y]	CB	Y	G	PCB	CB	Y
Treatment	Sucrose (mg • g^{-1} albedo FWT)						
Non-treated	19.9[x]	9.3	8.5	20.3	24.2	19.4	14.1
GA_3	18.4	13.7	11.9	16.9	23.1	21.7	23.7
Significance[w]	0.65	0.04	0.06	0.32	0.67	0.52	0.10

[z]Fruit developmental stage was a qualitative stage based on the appearance of non-treated fruit at each harvest.
[y]Harvest dates were as follows; G = 2 d after treatment (DAT), 2000 and 2001; PCB = 7 DAT, 2001; CB = 12, 2000, and 21, 2001, and Y = 54 DAT, 2000, and 58 DAT, 2001.
[x]Values are treatment means based on a sample of five fruit from each of three (2000 and Y, 2001) or four (2001) trees per treatment.
[w]$P \leq F$, as determined by a t-test within columns.

However, there was no apparent relationship between albedo sugar levels and sugar composition or SSC concentration of the juice (data not shown).

Conclusions. Gibberellic acid treatment before or after color break helped maintain green peel color, as expected. Gibberellic acid also considerably reduced fructose and glucose levels of the flavedo and fructose levels of the albedo. There was a consistent, inverse relationship between fructose and glucose, but not sucrose, levels of the flavedo and green peel color suggesting that GA_3 might modulate peel color by regulating peel hexose levels. Gibberellic acid treatment reduced juice SSC in 2001, but neither juice SSC nor the levels of individual juice sugars were apparently related to sugar levels of the flavedo or albedo.

CHAPTER 8
CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

Conclusions

Most Florida oranges are grown for processing. The value of fruit for processing increases in proportion to the fruit's soluble solids yield, the product of juice yield and Brix. Therefore, the citrus industry could benefit from practices that increase juice yield. Application of GA_3 in the fall can increase juice yield of oranges, but results are inconsistent and the reason why GA_3 application could increase yield is unknown. Thus, research was conducted to test the effect of variables that might influence GA_3 efficacy. In addition, effects of GA_3 application on mechanical and physiological properties of oranges were examined to determine if they were related to juice yield or Brix.

Rainfall may occur daily during the late summer and fall, when Florida growers might apply GA_3. An experiment was conducted to determine whether simulated rainfall ('wash-off' water sprays) might remove or dilute the compound before it has been fully absorbed, thus reducing efficacy. In 1998-99, peel hue angle and PPR of 'Hamlin' orange fruit increased linearly for ca. 1 h until wash-off and then reached a plateau, while in 1999-2000, peel hue angle and PPR increased linearly for ca. 2 h until wash-off before reaching a plateau. Therefore, rainfall within 1 to 2 h of application may reduce GA_3 effectiveness, even when a surfactant is used.

Preliminary research suggested that the inconsistent effect of GA_3 on juice yield might be related to application timing. Therefore an experiment was conducted to determine the optimal time to apply GA_3 for increasing juice yield of 'Hamlin',

'Pineapple' and 'Valencia' sweet oranges. Generally, the earliest application dates were most effective at maintaining peel puncture resistance above that of control fruit, while the latest application dates resulted in the most green peel color at each harvest. Juice yield of 'Hamlin' and 'Valencia', but not 'Pineapple', was increased by GA_3 at some application timings and harvest dates in both years. The increase in juice yield was related to time between application and harvest; juice yield of 'Hamlin' was greatest about 2 months, and 'Valencia' about 5 months after GA_3 application. Treated fruit often had lower juice Brix than non-sprayed fruit, a phenomenon that often paralleled treatment effects on peel color. When treatments did not increase juice yield but reduced juice Brix, then yield of solids was sometimes lower than for non-treated fruit. Treatments generally delayed flowering of 'Pineapple' and 'Valencia' but not 'Hamlin', possibly related to harvest date.

Some processors believe that GA_3 application increases mechanical integrity of the peel or fruit and thus improves the efficiency of juice extraction. To test this hypothesis, effects of GA_3 treatment on the mechanical properties of 'Valencia' orange peels and fruits were tested. The magnitude of peel and whole-fruit mechanical properties decreased between harvests, but more treatment effects were observed on the second harvest than on the first. Treatments affected more mechanical properties of the peel than the whole fruit. Mechanical properties of the peel were poorly correlated with thickness, probably because the mechanical properties of the tissues composing the peel are not equivalent. The earliest GA_3 application date was the most effective. On the second harvest, the peel of fruit treated before color break had 10% greater rupture force (in shear) and 15% greater tensile strength than the peel of non-treated fruit, whereas, the

properties of treated and non-treated whole fruits were similar. On the later harvest, the peel of fruit treated with GA_3 before color break had 10% greater rupture force than the peel of non-treated fruit, while the peel of fruit treated at color break had 15% greater shear strength than non-treated fruit. The peel of treated fruit generally had tensile properties that were 10-20% greater than the peel of control fruit, especially on the second harvest. Fruit treated before color break also had greater apparent modulus of elasticity and higher cutting force than other fruit. However, there was no obvious relationship between the mechanical properties measured and juice yield.

If GA_3 application does not improve juice extraction efficiency, then it must increase the proportion of juice in the fruit. Therefore, the effect of GA_3 treatment on peel growth and juice yield was tested. A secondary objective was to test whether the gap width, the distance between the cut edges of the peel of fruit sliced half way through the equator, might be a useful indicator of GA_3 efficacy and juice yield.

Green peel color of fruit treated with GA_3 was lost at half the rate of non-treated fruit. Application of GA_3 slightly increased juice yield 58 d after treatment (DAT). However, GA_3 also decreased percent peel volume between 35 and 77 DAT and percent peel volume was inversely related to juice yield ($r^2 = 0.54$). In addition, GA_3 treatment decreased juice Brix on 35 and 58 DAT, countering the positive effects of GA_3 on juice yield. Gap widths were not related to juice yield but they were significantly increased by GA_3 application and thus might be useful indicators of GA_3 efficacy.

Gibberellic acid applied to 'Hamlin' sweet oranges in the late summer and fall delays chloroplast degradation and development of yellow peel color (color break). Color break is preceded by an increase in peel soluble sugar levels, and sucrose feeding

studies have shown that high levels of soluble sugars stimulate color break. Therefore, the effects of a pre-color break GA_3-treatment on peel color and soluble sugar levels, and fruit respiration were tested.

Treatment effects on peel color were evident within 12 (2000) or 21 (2001) DAT. Treated and non-treated fruit had similar fruit respiration on most dates. Fructose, glucose and sucrose levels of flavedo and fructose and glucose levels of the albedo generally increased in both treated and non-treated fruit at each harvest date. However, treated fruit generally had lower flavedo and albedo fructose and glucose levels than non-treated fruit on days when treatment effects on peel color were evident. On these dates, there was a clear, negative relationship between green peel color and flavedo fructose and glucose levels (r^2=0.62, 2000 and 0.72, 2001). In contrast, there was only a moderate or no relationship between flavedo sucrose levels and green peel color in 2000 (r^2=0.49) and 2001 (r^2 = 0). Gibberellic acid effects on peel carbohydrate levels were not correlated with whole fruit respiration. Therefore, GA_3 treatment resulted in lower peel hexose levels and more green peel color, consistent with the hypothesis that glucose and fructose levels stimulate genes coding for suppression of photosythesis.

In conclusion, GA_3 application at about color break increased juice yield of oranges. However, GA_3 application also decreased juice Brix on some dates so solids yield was not increased. Gibberellic acid applied before color break increased the mechanical strength of orange peel but not of the whole fruit, and there was no apparent relationship between the mechanical properties measured and juice yield. Alternatively, application of GA_3 decreased peel thickness and thus peel volume. Because peel volume was inversely related to juice yield, GA_3 appears to increase juice yield by decreasing

peel volume. How GA_3 application decreased juice Brix remains unclear; however, it appears that GA_3 application maintains green peel color by suppressing levels of soluble sugars in the peel, especially fructose and glucose.

Suggestions for Future Research

The effect of GA_3 application on variables related to the quality of fruit for processing was investigated. However, these data are insufficient to determine whether application of GA_3 affects the yield of juice or soluble solids on the scale of the grove because effects of GA_3 on fruit loss or damage during on tree storage, harvest, and transport to the juice extractor, are unknown. Therefore, it is desirable to measure directly soluble solids yield of groves treated with GA_3 or non-treated.

Mechanical properties measured were not obviously related to juice yield, suggesting that GA_3 application did not improve extraction efficiency. However, the whole fruit tests were non-failure tests and thus might not be expected to relate closely with a destructive process such as juice extraction. Therefore, whole-fruit failure tests might be desirable.

Application of GA_3 appeared to maintain green peel color by suppressing accumulation of soluble peel hexoses. If this is true, then application of GA_3 probably affects the activity of enzymes related to sugar metabolism, a hypothesis that could be tested. In addition, the effect of GA_3 on sucrose metabolism could be measured directly by feeding radio labeled sucrose to cultured peel discs.

APPENDIX A
PERCENT JUICE YIELD AND PEEL PUNCTURE RESISTANCE OF 'HAMLIN' ORANGES

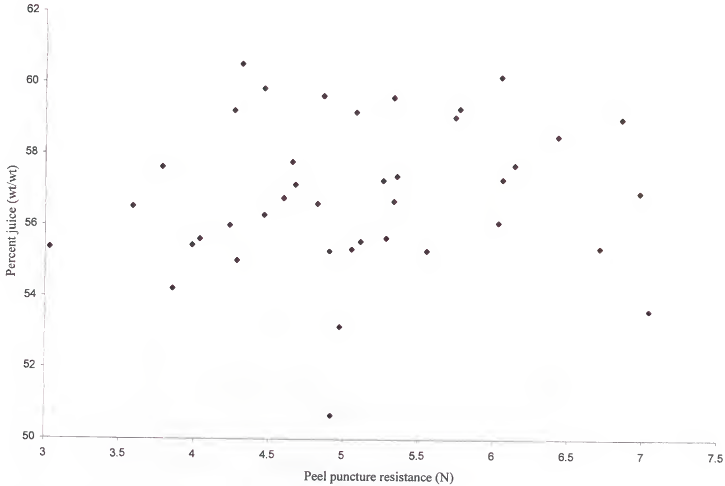

Fig. A. Percent juice yield and peel puncture resistance of 'Hamlin' oranges, Gainesville Fla., 2000.

APPENDIX B
EFFECT OF GIBBERELLIC ACID TREATMENT ON JUICE BRIX

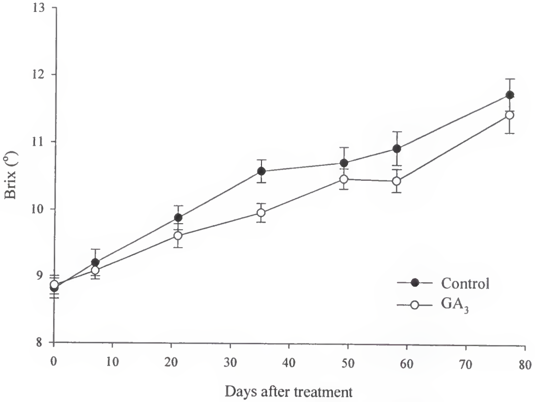

Fig. B. Effect of 2 Oct. 2001 GA_3 application on mean juice Brix of 'Hamlin' oranges, Groveland, Fla. Means $\pm$ SE, n=10 for each harvest date.

APPENDIX C
EFFECT OF GIBBERELLIC ACID APPLICATION ON RESPIRATION OF 'HAMLIN' ORANGE FRUIT

Table C. Respiration of 'Hamlin' orange fruit treated with GA_3 before or after colorbreak, or non-treated, and harvested 2, 12, 14, 21, 54 or 58 days after treatment, 2000, Gainesville, Fla. and 2001, Groveland, Fla.

Treatment	2000			2001				
	Days after treatment							
	2	12	54	2	7	14	21	58
	mg $CO_2 \bullet kg^{-1} \bullet h^{-1}$							
Pre color break								
GA_3	5.52[z]	5.93	6.87	6.03	3.69	-	2.99	5.33
Non-treated	6.00	7.27	6.93	5.02	3.44	-	3.05	6.08
Significance	NS	*	NS	*	NS	-	NS	NS
Post color break								
GA_3	5.17	6.23	-	-	-	5.16	-	-
Non-treated	5.94	7.62	-	-	-	4.9	-	-
Significance	NS	*	-	NS	-	NS	-	-

[z]Values are treatment means. Respiration of a five-fruit sample was measured for each of four branches/treatment. NS, *, Non-significant or significant at $P \leq 0.05$, respectively.

LIST OF REFERENCES

Agusti, M., V. Almela, and J. L. Guardiola. 1981. The regulation of fruit cropping in mandarins through the use of growth regulators. Proc. Intl. Soc. Citricult. 216-220.

Ahmed, E. M., F. G. Martin, and R. C. Fluck. 1973. Damaging stresses to fresh and irradiated citrus fruit. J. Food Sci. 38:230-233.

Albrigo, L. G. and R. D. Carter. 1977. Structure of citrus fruits in relation to processing, p. 33-73. In: S. Nagy, P. E. Shaw, and M. K. Veldhuis (eds.). Citrus science and technology. AVI Publ. Co., Westport, Conn.

Altman, A., Y. Gulsen, and R. Goren. 1982. Growth and metabolic activity of lemon juice vesicle explants in vitro. Plant Physiol. 69:1-6.

Anonymous. 2001. Florida citrus summary 2000-1999. Florida Agr. Stat. Ser., Orlando, Fla.

ASAE. 1984. Compression tests of food materials of convex shape. ASAE Standard 5368.1 ASAE Standards 1984. ASAE, St. Joseph, MI 49085.

Baldwin, E. A., M. O. Nisperos-Carriedo, and M. G. Moshonas. 1991. Quantitative analysis of flavor and other volatiles and for certain constituents of two tomato cultivars during ripening. J. Amer. Soc. Hort. Sci. 116:265-269.

Baur, P., A. Bucholz, and J. Schonner. 1997. Diffusion in plant cuticles as affected by temperature and size of organic solutes: similarity and diversity among species. Plant Cell Envir. 20:982-994.

Berry, R. E. and M. K. Veldhuis. 1977. Processing of oranges, grapefruit and tangerines, p. 190-191. In S. Nagy, P. E. Shaw, and M. K. Veldhuis (eds.). Citrus science and technology. AVI Publ. Co., Westport, Conn.

Braddock, R. J. 1999. Handbook of citrus by-products and processing technology. Wiley-Interscience, N.Y.

Cary, P. R. 1975. Citrus fruit maturity. Austral. Citrus News, April:10.

Churchill, D. B., H. R. Sumner, and J. D. Whitney. 1980. Peel strength properties of three orange varieties. Trans. ASAE: 173-176.

Coggins, C. W. 1969. Gibberellin research on citrus rind aging problems. Proc. First Intl. Citrus Symp. 3:1177-1185.

Coggins, C. W. 1981. The influence of exogenous growth regulators on rind quality and internal quality of citrus fruits. Proc. Intl. Soc. Citricult. 214-216.

Coggins, C. W. and G. L. Henning. 1988. A comprehensive California field study of the influence of preharvest application of gibberellic acid on the rind quality of Valencia oranges. Israel J. Bot. 37:145-154.

Coggins, C. W. and H. Z. Hield. 1958. Gibberellin on orange fruit. Calif. Agr. 12:11.

Coggins, C. W. and H. Z. Hield. 1968. Plant growth regulators, p. 371-389. In: W. Reuther, L. D. Batchelor, and H. J. Webber (eds.). The citrus industry, Vol. II. Univ. of Calif. Berkeley.

Coggins, C. W. and W. W. Jones. 1977. Growth regulators and coloring of citrus fruits. Proc. Intl. Soc. Citricult. 2:686-688.

Coggins, C. W. and L. N. Lewis 1965. Some physical properties of the navel orange rind as related to ripening and to gibberellic acid treatments. Proc. Amer. Soc. Hort. Sci. 86:272-279.

Coggins, C. W., G. L. Henning, and M. F. Anthony. 1992. Possible methods to increase efficacy of gibberellic acid applied to navel orange trees, p. 567-572. In: C. Foy (ed.). Adjuvants for agrichemicals. CRC Press, Boca Raton, Fla.

Coggins, C. W., G. L. Henning, and D. R. Atkin. 1974. Gibberellic acid compatability studies on citrus. J. Amer. Soc. Hort. Sci. 99:197-199.

Coggins, C. W., H. Z. Hield, and R. M. Burns. 1962. The influence of potassium gibberellate on grapefruit trees and fruit. Proc. Amer. Soc. Hort. Sci. 81:223-26.

Coggins, C. W., H. Z. Hield, and M. J. Garber. 1960a. The influence of potassium gibberellate on Lisbon lemon trees and fruit. Proc. Amer. Soc. Hort. Sci. 76:199-207.

Coggins, C. W., H. Z. Hield and M. J. Garber. 1960b. The influence of potassium gibberellate on Valencia orange trees and fruit. Proc. Amer. Soc. Hort. Sci. 76:193-98.

Coggins, C. W., H. Z. Hield, I. L. Eaks, L. N. Lewis, and R. M. Burns. 1965. Gibberellin research on citrus. Calif. Citrogr. 50: 457, 466-468.

Davenport, T. L. 1990. Citrus flowering, p. 349-408. In: Janick, J. (ed.). Hort. Rev. Vol. 13. Timber Press, Portland, OR.

Davies, F. S. 1986. Growth regulator improvement of postharvest quality, p. 79-99 In: Fresh citrus fruit. W. Wardowski, S. Nagy, W. Grierson (es.). AVI Press, Westport, Conn.

Davies, F. S. and L. G. Albrigo. 1994. Citrus. CAB Intl., Wallingford Oxon, U.K.

Davies, F. S., C. A. Campbell, and G. Zalman. 1997. Gibberellic acid sprays for improving fruit peel quality and increasing juice yield of processing oranges. Proc. Fla. State. Hort. Soc. 110:16-21.

Davies, F. S., C. A. Campbell, and M. W. Fidelibus. 2001. Gibberellic acid tank mix and adjuvant effects on peel quality and juice yield of 'Hamlin' oranges. HortTechnology 11:171-174.

Davies, F. S., C. A. Campbell, G. Zalman, and M. Fidelibus. 1999. Gibberellic acid application timing effects on juice yield and peel quality of 'Hamlin' oranges. Proc. Fla. State Hort. Soc. 112:22-24.

Davies, P. J. 1995. The plant hormones: their nature, occurrence, and functions, p. 1-12 In: P. J. Davies (ed.). Plant hormones: physiology, biochemistry and molecular biology. 1995. Kluwer Acad. Publ., Boston, Mass.

DeWald, D. B., A. Sadka, and J. E. Mullet. 1994. Sucrose modulation of soybean vsp gene expression is inhibited by auxin. Plant Physiol. 104:439-444.

Dinar, H. M. A., A. H. Krezdorn, and A. J. Rose. 1976. Extending the grapefruit harvest season with growth regulators. Proc. Fla. State Hort. Soc. 89:4-6.

Eaks, I. L. 1970. Respiratory response, ethylene production, and response to ethylene of citrus fruit during ontogeny. Plant Physiol. 45:334-338.

Eilati, S. K., E. E. Goldschmidt, and S. P. Monselise. 1969. Hormonal control of color changes in orange peel. Experimentia 15: 209-210.

El-Otmani, M. and C. W. Coggins. 1985. Fruit age and growth regulator effects on the quantity and structure of the epicuticular wax of 'Washington' navel orange fruit. J. Amer. Soc. Hort. Sci. 110:371-378.

El-Otmani, M., C. W. Coggins, and I. L. Eaks. 1986. Fruit age and gibberellic acid effect on epicuticular wax accumulation, respiration, and internal atmosphere of navel orange fruit. J. Amer. Soc. Hort. Sci. 111:228-232.

El-Otmani, M. and C. W. Coggins. 1991. Growth regulator effects on retention of quality of stored citrus fruits. Scientia Hort. 45:261-272.

El-Otmani, M., C. J. Lovatt, C. W. Coggins, and M. Agusti. 1995. Plant growth regulators in citriculture: factors regulating endogenous levels in citrus tissues. Crit. Rev. Plant Sci. 14:367-412.

El-Otmani, M., C. W. Coggins, M. Agusti, and C. J. Lovatt. 2000. Plant growth regulators in citriculture: world current uses. Crit. Rev. Plant Sci. 14:367-412.

Erickson, L. C. 1968. The general physiology of citrus, p. 86-126. In: W. Reuther, L. D. Batchelor, and H. J. Webber (eds.). The citrus industry, Vol. II. Univ. of Calif. Berkeley.

Erner, Y., R. Goren, and S. P. Monselise. 1976. The induction of peel roughness of 'Shamouti' orange with growth regulators. J. Amer. Soc. Hort. Sci. 101:513-515.

Ferguson, L. 1984. Gibberellic acid: translocation, metabolism and effects on peel quality of 'Marsh' grapefruit (*Citrus paradisi* Macf.). Ph.D. thesis, Dept. Hort. Sci., Univ. Fla., Gainesville.

Ferguson, L., T. A. Wheaton, F. S. Davies and M. A. Ismail. 1986. ^{14}C-gibberellic acid uptake, translocation, persistence, and metabolism in grapefruit. J. Amer. Soc. Hort. Sci. 111:926-932.

Fidelibus, M. W., A. A. Teixeira, and F. S. Davies. 2002a. Mechanical properties of orange peel and fruit treated pre-harvest with gibberellic acid. Trans. ASAE. (in press).

Fidelibus, M. W., F. S. Davies, and C. A. Campbell. 2002b. Gibberellic acid application timing affects fruit quality of processing oranges. HortScience 37:353-357.

Fucik, J. E. 1981. The effect of preharvest foliar sprays of 2,4-D and gibberellic acid on the postharvest storage characteristics of 'ruby red' grapefruit. Proc. Intl. Soc. Citricult. 221-224.

Garcia-Luis, A., M. Agusti, V. Almela, E. Romero, and J. L. Guardiola. 1985. Effect of gibberellic acid on ripening and peel puffing in 'Satsuma' mandarin. Scientia Hort. 27:75-86.

Garcia-Luis, A., V. Almela, C. Monerri, M. Agusti, and J. L. Guardiola. 1986. Inhibition of flowering in vivo by existing fruits and applied growth regulators in *Citrus unshiu*. Physiol. Plant. 66:515-520.

Garcia-Luis, A., A. M. M. Duarte, M. Kanduser, and J. L. Guardiola. 2001. The anatomy of the fruit in relation to the propensity of citrus species to split. Scientia Hort. 87:33-52.

Gillfillan, I. M. 1986. Acidification of gibberellic acid sprays on citrus trees. Citrus Subtrop. Fruit J. 8:628.

Gillfillan, I. M. and J. G. M. Cutting. 1992. Creasing reduction in navel oranges: lower efficacy of gibberellic acid spray mixtures containing petroleum oil. Proc. Intl. Soc. Citricult. 527-529.

Goldschmidt, E. E. 1983. Asymetric growth of citrus fruit peel induced by localized application of gibberellins in lanolin pastes. J. Hort. Sci. 29-35.

Goldschmidt, E. E. and D. Galili. 1974. The fate of endogenous gibberellins and applied radioactive gibberellin A_3 during natural and ethylene-induced senescence in citrus peel. Plant Cell Physiol. 15:485-491.

Goldschmidt, E. E. and D. Galili. 1981. Fate of ^{14}C-gibberellic acid in senescing on-tree 'Valencia' orange fruit. J. Amer. Soc. Hort. Sci. 106:175-177.

Goldschmidt, E. E. and K. E. Koch. 1996. Citrus, p. 797-823. In: E. Zamski and A. A. Schaffer (eds.). Photoassimilate distribution in plants and crops. Marcel Dekker, N.Y.

Goldschmidt, E. E. and S. P. Monselise. 1972. Hormonal control of flowering in citrus and some other woody perennials, p. 758-766. In: D. J. Carr (ed.) Plant growth substances. Springer Verlag, Berlin.

Greenberg, J. and E. E. Goldschmidt. 1988. The effectiveness of GA_3 application to citrus fruit. Proc. 6th Intl. Citrus Congr. 2:339-342.

Greenberg, J. and E. E. Goldschmidt. 1989. Acidifying agents, uptake, and physiological activity of gibberellin A_3 in *Citrus*. HortScience 24:791-793.

Greenberg, J. and E. E. Goldschmidt. 1990. Uptake of ^{14}C gibberellin by mature grapefruit (*Citrus paradisi* Macf.) as affected by relative humidity and method of application. Ann. Applied Biol. 116:383-390.

Greenberg, J., E. E. Goldschmidt, S. Schechter, S. P. Monselise, and D. Galili. 1984. Improving the uptake of gibberellic acid (GA_3) by citrus fruit and leaves. Proc. XI Annu. Mtg. Plant Growth Regulat. Soc. Amer.: 16-25.

Greenberg, J. S. P. Monselise, and E. E. Goldschmidt. 1987. Improvement of gibberellin efficiency in prolonging the citrus harvest season by the surfactant L-77. J. Amer. Soc. Hort. Sci. 112:625-629.

Greenberg, J. S., Y. Oren, G. Eshel, and E. E. Goldschmidt. 1992. Gibberellin A_3 (GA_3) on 'Minneola' tangelo; extension of the harvest season and improvement of fruit quality. Proc. Intl. Soc. Citricult. 7:456-458.

Gregoriou, C. A., Gregoriou, A., and F. S. Davies. 1996. Growth regulator, fruit drop and water spot of mandarins in Cyprus. Agr. Res. Inst. Tech. Bul. 175, Nicosia, Cyprus.

Guardiola, J. L., M. Agusti, and F. Garcia-Mari. 1977. Gibberellic acid and flower bud development in sweet orange. Proc. Intl. Soc. Citricult. 2:696-699.

Guardiola, J. L., M. T. Barres, C. Albert, and A. Garcia-Luis. 1993. Effects of exogenous growth regulators on fruit development in *Citrus unshiu*. Ann. Bot. 71:169-176.

Gyasi, S. R., B. Friedly, and P. Chen. 1981. Elastic and viscoelastic Poisson's ratio determination for selected citrus fruits. Trans. ASAE 24:747-750.

Harada, H., H. Mukai, and T. Takagi. 2001. Effects of explant age, growth regulators and carbohydrates on sugar accumulation in *Citrus* juice vesicles cultured in vitro. Scientia Hort. 90:109-119.

Harker, F., R. Redgwell, I. Hallett, S. Murray, and G. Carter. 1997. Texture of fruit flesh, p. 121-223. In: Janick, J. (ed.). Hort. Rev. Vol. 20. Timber Press, Portland, OR.

Hedden, P. and Y. Kamiya. 1997. Gibberellin biosynthesis: enzymes, genes and their regulation. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:431-60.

Heldt, H-W. Plant biochemistry and molecular biology. Oxford Univ. Press, Oxford, U.K.

Henning, G. L. and C. W. Coggins, 1988. Bioassay used to determine the impact of surfactants on the biological effectiveness of exogenous gibberellic acid. Proc. Sixth Intl. Citrus Congr. 325-331.

Huff, A. 1984. Sugar regulation of plastid interconversions in epicarp of citrus fruit. Plant Physiol. 76:307-312.

Iglesias, D. J., F. R. Tadeo, F. Legaz, E. Primo-Millo, and M. Talon. 2001. In vivo sucrose stimulation of colour change in citrus fruit epicarps: interactions between nutritional and hormonal signals. Physiol. Plant. 112:244-250.

Kaufmann, M. R. 1970. Extensibility of pericarp tissue in growing citrus fruits. Plant Physiol. 46:778-781.

Kende, H. and J. A. D. Zeevardt. 1997. The five "classical" plant hormones. Plant Cell 9:1197-1210.

King, R. W. and Y. Ben-tal. 2001. A florigenic effect of sucrose in *Fuchsia hybrida* is blocked by gibberellin-induced assimilate competition. Plant Physiol. 125:488-496.

Koch, K. E. 1996. Carbohydrate modulated gene expression in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 47:509-540.

Koch, K. E. and W. T. Avigne. 1990. Postphloem, nonvascular transfer in citrus: kinetics, metabolism, and sugar gradients. Plant Physiol. 93:1405-1416.

Komatsu, A., T. Moriguchi, K. Koyama, M. Omura, and T. Akihama. 2002. Analysis of sucrose synthase genes in citrus suggests different roles and phylogenetic relationships. J. Exp. Bot. 53:61-71.

Krezdorn, A. H. and M. Cohen. 1962. The influence of chemical fruit set sprays on yield and quality of citrus. Proc. Fla. State Hort. Soc. 75:53-60.

Kurosawa, E. 1926. Experimental studies on the nature of the substance excreted by the "bakane" fungus. Trans. Nat. Hist. Soc. Formosa. 16:213-227.

Lewis, L. N., C. W. Coggins, and H. Z. Hield. 1964. The effect of biennial bearing and NAA on the carbohydrate composition of Wilking mandarin leaves. Proc. Amer. Soc. Hort. Sci. 84:147-151.

Lewis, L. N., C. W. Coggins, C. K. Labanauskas, and W. M. Dugger. 1967. Biochemical changes associated with natural and gibberellin A_3 delayed senescence in the navel orange rind. Plant Cell Physiol. 8:151-160.

Martin, G. C. 1983. Commercial uses of gibberellins, p. 395-444. In: A. Crozier, ed. The biochemistry and physiology of gibberellins. Praeger, N.Y.

McDonald, R. E., P. E. Shaw, P. D. Greany, T. T. Hatton, and C. W. Wilson. 1987. Effect of gibberellic acid on certain physical and chemical properties of grapefruit. Trop. Sci. 27:17-22.

Miller, W. M. 1986. Physical properties data for postharvest handling of Florida citrus. Appl. Eng. Agr. 3:123-128.

Monselise, S. P. 1977. Citrus fruit development: endogenous systems and external development. Proc. Intl. Soc. Citricult. 2:664-668.

Monselise, S. P. and A. H. Halevy. 1964. Chemical inhibition and promotion of citrus flower bud induction. Proc. Amer. Soc. Hort. Sci. 84:141-46.

Monselise, S. P. and R. Goren. 1965. Changes in composition and enzymatic activity in flavedo of Shamouti oranges during the color break period, as influenced by application of gibberellin and 2-chloroethyl trimethyl-ammonium chloride. Phyton 22:61-66.

Monselise, S. P., M. Weiser, N. Shafir, R. Goren, and E. E. Goldschmidt. 1976. Creasing of orange peel-physiology and control. J. Hort. Sci. 51:341-351.

Moss, G. I. and J. Bellamy. 1972. The use of gibberellic acid to control flowering in sweet orange. Acta Hort. 34:207-212.

Muramatsu, N., T. Takahara, T. Ogata, and K. Kojima. 1999. Changes in rind firmness and cell wall polysaccharides during citrus fruit development and maturation. HortScience 34:79-81.

Nagar, P. K. 1994. Effect of some ripening retardents on fruit softening enzymes of kinnow mandarin fruits. Indian J. Plant Physiol. 37:122-124.

Phinney, B. O. 1983. The history of gibberellins, p. 19-52. In: A. Crozier (ed.). The biochemistry and physiology of gibberellins. Praeger, N.Y.

Pozo, L., W. J. Kender, J. K. Burns, U. Hartmond, and A. Grant. 2000. Effects of gibberellic acid on ripening and rind puffing in 'Sunburst' mandarin. Proc. Fla. State Hort. Soc. 113:102-105.

Purvis, A. C. and J. D. Rice. 1983. Low temperature induction of invertase activity in grapefruit flavedo tissue. Phytochemistry 22:831-834.

Rasmussen, G. K. 1973. The effect of growth regulators on degreening and regreening of citrus fruit. Acta Hort. 34:473-478.

Reeve, D. R. and A. Crozier. 1975. Gibberellin bioassays, p. 35-64. In: H. N. Krishnamoorthy (ed.). Gibberellins in plant growth. Wiley Eastern, New Delhi.

Ritenour, M. A. and E. Stover. 1999. Effects of gibberellic acid on the harvest and storage quality of Florida citrus fruit. Proc. Fla. State Hort. Soc. 112:122-125.

Rokach, A. 1953. Water transfer from fruits to leaves in Shamouti orange tree and related topics. Palestine J. Bot. 8:146-151.

Sarig, Y. and D. Nahir. 1973. Deformation characteristics of 'Valencia' oranges as an indicator of firmness. HortScience 8:391-392.

Schneider, H. 1968. The anatomy of citrus, p. 1-85. In: W. Reuther, L. D. Batchelor, and H. J. Webber (eds.). The citrus industry, Vol II. Univ. of Calif. Press, Berkeley.

Sinclair, W. B. 1972. The grapefruit: its composition, physiology, and products. Univ. of Calif. Press, Berkeley.

Sites, J. W. and H. J. Reitz. 1950. The variation in individual 'Valencia' oranges from different locations on the tree as a guide to sampling methods and spot picking for quality. II. Titratable acid and the soluble solids/titratable acid ratio of the juice. Proc. Amer. Soc. Hort. Sci. 55:73-80.

Smith, V. A. 1992. Gibberellin A_1 biosynthesis in *Pisum sativum* L. II. Biological consequences of the *le* mutation. Plant Physiol. 99:372-377.

Spiegel-Roy, P. and E. E. Goldschmidt. 1996. The biology of citrus. Cambridge Univ. Press, Cambridge, U.K.

Sponsel, V. M. 1995. The biosynthesis and metabolism of gibberellins in higher plants. p. 66-97. In: P. J. Davies (ed.). Plant hormones: physiology, biochemistry, and molecular biology. Kluwer Acad. Publ., Boston, Mass.

Thompson, W. W., L. N. Lewis, and C. W. Coggins. 1967. The reversion of chromoplasts to chloroplasts in Valencia oranges. Cytologia 32:117-124.

Wardowski, W., J. Whigham, W. Grierson, and J. Soule. 1995. Quality tests for Florida citrus. Univ. of Florida Coop. Ext. Serv. SP99.

Wilson, W. C. 1983. The use of exogenous plant growth regulators on citrus. Plant Growth Regul. Chem.:207-232.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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